BB

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/705	A1	(11) International Publication Number: WO 97/17368
		(43) International Publication Date: 15 May 1997 (15.05.97)

(21) International Application Number: PCT/US96/18645

(22) International Filing Date: 12 November 1996 (12.11.96)

(30) Priority Data:
08/556,422 9 November 1995 (09.11.95) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/556,422 (CIP)
Filed on 9 November 1995 (09.11.95)

(71) Applicant *(for all designated States except US)*: DANA-FARBER CANCER INSTITUTE [US/US]; 44 Binney Street, Boston, MA 02115 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: HALL, Kathryn, T. [JM/US]; 15 Leland Street #1, Boston, MA 02130 (US). FREEMAN, Gordon, J. [US/US]; 305 Walnut Street, Brookline, MA 02146 (US). SCHULTZE, Joachim, L. [DE/US]; 37 Auburn Street, Brookline, MA 02146 (US). BOUSSIOTIS, Vassiliki, A. [GR/US]; Apartment #403, 44 Washington Street, Brookline, MA 02146 (US). NADLER, Lee, M. [US/US]; 36 Cross Hill Road, Newton, MA 02159 (US).

(74) Agents: MANDRAGOURAS, Amy, E. et al.; Lahive & Cockfield, 60 State Street, Boston, MA 02109 (US).

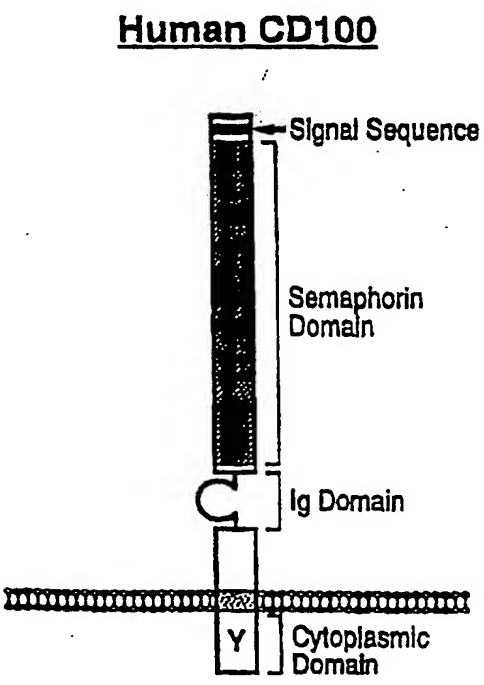
(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CD100 ANTIGEN AND USES THEREFOR

(57) Abstract

Isolated nucleic acid molecules encoding novel CD100 molecules which stimulate a leukocyte response, such as a B cell response, including B cell aggregation, B cell differentiation, B cell survival, and/or T cell proliferation are disclosed. These novel molecules have a certain homology to semaphorins, proteins which are growth cone guidance molecules that are critical for guiding growing axons of neurons to their targets. In addition to isolated nucleic acid molecules, antisense nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced are also described. The invention further provides isolated CD100 proteins, fusion proteins and active fragments thereof. Diagnostic and therapeutic methods utilizing compositions of the invention are also provided.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

CD100 ANTIGEN AND USES THEREFOR

Background of the Invention

Induction of a humoral response is important in host defense, for example, in fighting infections by pathogens. A humoral response is mediated by B cells, but requires the help of other cells, such as T cells. Development of a humoral response is a multistage process which occurs primarily in secondary lymphoid tissues.

Resting B cells circulate in the blood, pass through secondary lymphoid tissues, such as lymph nodes, Peyer's patches, spleen and tonsils, where they come into contact with trapped antigens. B cells recognizing a specific antigen through their surface immunoglobulins process the antigen and enter the T cell rich paracortical regions below the outer layer, or cortex, of lymphoid tissues. Some of the T cells in this region have been activated through contact with antigen presenting cells. These T cells in turn promote B cell activation by direct contact with the B cells via interaction of cell surface molecules and by the secretion of T cell-specific cytokines, such as IL-2, IL-4, and IL-5. This cell-cell interaction between T and B cells or production of T cell specific cytokines induces B cells to migrate into B cell follicles. In these follicles, the B cells interact with follicular dendritic cells having antigen-antibody complexes on their surface resulting in the formation of a germinal center. B cells in germinal centers undergo active proliferation, affinity maturation, and differentiation into memory B cells or antibody secreting plasma cells. Thus, whereas antibody-secreting plasma cells produce antibodies to fight infections, memory B cells assure a more rapid response to subsequent exposure to the same antigen.

Some molecules involved in the induction of B-cell proliferation and differentiation have been identified. In addition to cytokines produced in large part by activated T cells, crosslinking of specific B cell surface molecules also provide such signals (Clark E. A.et al, (1994), *Nature* 367, 425). One such B cell surface molecule is CD40. CD40 is a 45-50 kD protein expressed on activated B cells. Valle et al., (1989), *Eur. J. Immunol.*, 19:1463-1467; Gordon et al., (1988), *J. Immunol.*, 140:1425-1430; Gruber et al., (1989), *J. Immunol.*, 142: 4144-4152. Crosslinking of CD40 with antibodies or with its natural ligand, CD40L, also termed gp39, together with other stimulatory signals induces B cell proliferation and antibody production. Armitage et al., (1992), *Nature*, 357:80-82 ; Hollenbaugh et al., (1992), *EMBO J.*, 11:4313-4319.

T cells are not only required for providing help to B cells, but also play a major role in cellular immune responses, such as in delayed type hypersensitivity reactions and in cytotoxicity. To exert their activity, T cells must be activated. To induce antigen-specific T cell activation and clonal expansion, two signals provided by antigen-presenting cells (APCs) must be delivered to the surface of resting T lymphocytes (Jenkins, M. and Schwartz, R. (1987) *J. Exp. Med.* 165, 302-319; Mueller, D.L., et al. (1990) *J. Immunol.* 144, 3701-3709; Williams, I.R. and Unanue, E.R. (1990) *J. Immunol.* 145, 85-93). The

first signal, which confers specificity to the immune response, is mediated via the T cell receptor (TCR) following recognition of foreign antigenic peptide presented in the context of the major histocompatibility complex (MHC). The second signal, termed costimulation, induces T cells to proliferate and become functional (Schwartz, R.H. (1990) *Science* 248, 1349-1356). Costimulation is neither antigen-specific, nor MHC restricted and is thought to be provided by one or more distinct cell surface molecules expressed by APCs (Jenkins, M.K., et al. (1988) *J. Immunol.* 140, 3324-3330; Linsley, P.S., et al. (1991) *J. Exp. Med.* 173, 721-730; Gimmi, C.D., et al., (1991) *Proc. Natl. Acad. Sci. USA.* 88, 6575-6579; Young, J.W., et al. (1992) *J. Clin. Invest.* 90, 229-237; Koulova, L., et al. (1991) *J. Exp. Med.* 173, 759-762; Reiser, H., et al. (1992) *Proc. Natl. Acad. Sci. USA.* 89, 271-275; van-Seventer, G.A., et al. (1990) *J. Immunol.* 144, 4579-4586; LaSalle, J.M., et al., (1991) *J. Immunol.* 147, 774-80; Dustin, M.I., et al., (1989) *J. Exp. Med.* 169, 503; Armitage, R.J., et al. (1992) *Nature* 357, 80-82; Liu, Y., et al. (1992) *J. Exp. Med.* 175, 437-445). B7-1 and B7-2 are two such costimulatory molecules which interact with CD28 and CTLA4 on T cells (Linsley, P.S., et al., (1991) *J. Exp. Med.* 173, 721-730; Gimmi, C.D., et al., (1991) *Proc. Natl. Acad. Sci. USA.* 88, 6575-6579; Koulova, L., et al., (1991) *J. Exp. Med.* 173, 759-762; Reiser, H., et al. (1992) *Proc. Natl. Acad. Sci. USA.* 89, 271-275; Linsley, P.S. et al. (1990) *Proc. Natl. Acad. Sci. USA.* 87, 5031-5035; Freeman, G.J. et al. (1991) *J. Exp. Med.* 174,625-631; Freeman, G.J. et al. (1993) *Science* 262:909-911; Azuma, M. et al. (1993) *Nature* 366:76-79; and Freeman, G.J. et al. (1993) *J. Exp. Med.* 178:2185-2192). Though the molecules B7-1 and B7-2 play a critical role in costimulation of T cell, there is some evidence that additional molecules can provide a costimulatory signal to T cells.

Previous studies demonstrated that several antibodies recognized a 150kD cell-surface homodimer, termed CD100, that is expressed on a number of hematopoietic cells including B and T lymphocytes, granulocytes, monocytes and natural killer cells, but not on eosinophils, platelets, erythrocytes or hematopoietic progenitor cells (Bougeret, C. et al., (1992), *J. Immunol.* 148, 318; Herold, C., et al., Eds., (Oxford University Press, Oxford, 1995), *Leucocyte Typing V.*, S. F. Schlossman, et al., Eds. vol. 1, pp. 52). These studies indicated that CD100 expression on resting T cells increases on T cells after phytohemagglutinin activation (Bougeret, C. et al., (1992), *J. Immunol.* 148, 318). In addition, it has been reported that crosslinking of CD100 provides a costimulatory signal to T cells, indicating that this molecule may be involved in T cell activation and clonal expansion (Herold C. et al., (1994), *Int. Immunol.* 7, 1). However, the role of CD100, in particular its role on B lymphocytes is unknown.

Summary of the Invention

This invention provides isolated nucleic acid molecules encoding a CD100 antigen. Such nucleic acid molecules (e.g., cDNAs) have a nucleotide sequence encoding a CD100 antigen or biolocially active portions thereof, such as a peptide having a CD100 activity. In

a preferred embodiment, the isolated nucleic acid molecule has a nucleotide sequence shown in Figure 1, SEQ ID NO: 1, or a portion thereof such as the coding region of the nucleotide sequence of Figure 1, SEQ ID NO: 1. Other preferred nucleic acid molecules encode a protein having the amino acid sequence of Figure 2, SEQ ID NO: 2. In another preferred embodiment, the isolated nucleic acid molecule has a nucleotide sequence shown in Figure 12, SEQ ID NO: 8 and/or encodes a protein having an amino acid sequence shown in Figure 14, SEQ ID NO: 9. Nucleic acid molecules derived from hematopoietic cells (e.g., a naturally-occurring nucleic acid molecule found in an activated lymphocyte) which hybridize under stringent conditions to the nucleotide sequence shown in Figure 1, SEQ ID NO: 1 or Figure 12, SEQ ID NO: 8 are also within the scope of the invention.

In another embodiment, the isolated nucleic acid molecule is a nucleotide sequence encoding a protein having an amino acid sequence which is at least about 80%, preferably at least about 85%, more preferably at least about 90% and most preferably at least about 95-99% overall amino acid sequence identity with an amino acid sequence shown in Figure 2, SEQ ID NO: 2. This invention further pertains to nucleic acid molecules which encode a protein which includes a semaphorin domain having an amino acid sequence at least about 80%, preferably at least about 90%, more preferably at least about 95-99% identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2. Also within the scope of this invention are nucleic acid molecules which encode a protein which includes an extracellular domain having an amino acid sequence at least 80%, preferably at least 90%, more preferably at least 95-99% identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2.

Nucleic acid molecules encoding proteins which include a semaphorin domain having an amino acid sequence at least about 60% (preferably at least about 70%, 80%, 90%, or 95-99%) identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2 and an immunoglobulin-like domain having an amino acid sequence at least about 50% (preferably at least about 60%, 70%, 80%, 90%, or 95-99%) identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2 are also within the scope of this invention. These nucleic acid molecules can encode proteins which optionally include a cytoplasmic domain having an amino acid sequence at least about 50% (preferably at least about 60%, 70%, 80%, 90%, or 95-99%) identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2.

Another aspect of this invention pertains to nucleic acid molecules encoding a CD100 fusion protein which includes a nucleotide sequence encoding a first peptide having an amino acid sequence at least about 80% (preferably at least about 90%, or 95-99%) identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2 and a nucleic sequence encoding a second peptide c rresponding to a moiety that facilitates detection or purification of the molecules or that alter the solubility, binding affinity or valency of the first peptide, such as an immunoglobulin constant region.

In another embodiment, the isolated nucleic acid molecule has a nucleotide sequence encoding a peptide fragment of at least about 30 amino acid residues in length, preferably at least about 40 amino acid residues in length, and more preferably at least about 50 amino acid residues in length corresponding to an amino acid sequence shown in Figure 2, SEQ ID NO: 2. In a preferred embodiment, the peptide fragment has a CD100 activity.

Moreover, given the disclosure herein of a CD100-encoding cDNA sequence (e.g., SEQ ID NO: 1), antisense nucleic acid molecules (i.e, molecules which are complimentary to the coding strand of the CD100 cDNA sequence) are also provided by the invention.

Another aspect of the invention pertains to recombinant expression vectors containing the nucleic acid molecules of the invention and host cells into which such recombinant expression vectors have been introduced. In one embodiment, such a host cell is used to produce CD100 protein by culturing the host cell in a suitable medium. If desired, CD100 protein can then be isolated from the medium or the host cell.

Still another aspect of the invention pertains to isolated CD100 proteins and active fragments thereof, such as peptides having an activity of a CD100 antigen (e.g., at least one biological acitivity of CD100, such as the ability to stimulate a B cell response, for example, B cell aggregation or the ability to stimulate a T cell response, for example, T cell proliferation). The invention also provides an isolated preparation of a CD100 protein. In preferred embodiments, the CD100 protein comprises an amino acid sequence of Figure 2, SEQ ID NO: 2, or a mature CD100 protein lacking an amino-terminal signal sequence (e.g., a CD100 molecule having amino acid residues 22-862 or 24-862 of Figure 2, SEQ ID NO: 2 or having amino acid residues 22-861 or 24-861 of Figure 14, SEQ ID NO: 9). In other embodiments, the isolated CD100 protein comprises an amino acid sequence at least 80 % identical to an amino acid sequence of Figure 2, SEQ ID NO: 2 and, preferably has an activity of CD100 (e.g., at least one biological activity of CD100). Preferably, the protein is at least about 90 %, more preferably at least about 95 %, even more preferably at least about 98 % and most preferably at least about 99 % identical to the amino acid sequence of Figure 2, SEQ ID NO: 2.

This invention also pertains to isolated peptides which include a semaphorin domain having an amino acid sequence that is at least about 60% (preferably at least about 70%, 80%, 90%, or 95-99%) identical to an amino acid sequence shown in Figure 2 (SEQ ID NO: 2) and an immunoglobulin-like domain having an amino acid sequence that is at least 50% (preferably at least about 60%, 70%, 80%, 90%, or 95-99%) identical to an amino acid sequence shown in Figure 2 (SEQ ID NO: 2). These peptides can optionally include a cytoplasmic domain having an amino acid sequence that is at least about 50% identical to an amino acid sequence shown in Figure 2 (SEQ ID NO: 2).

The invention also provides for a CD100 fusion protein comprising a first peptide having an amino acid sequence at least about 80% identical to an amino acid sequence shown in Figure 2 (SEQ ID NO: 2) and a second peptide corresponding to a moiety that alters the solubility, binding affinity or valency of the first peptide. In preferred embodiments, the fusion protein comprises an extracellular domain of a CD100 antigen. In yet another embodiment, the fusion protein comprises a semaphorin domain of a CD100 antigen.

Peptides comprising a fragment of at least about 30 amino acids, a fragment of at least about 40 amino acids, a fragment of at least about 50 amino acids, or longer fragments of the sequence shown in Figure 2 (SEQ ID NO: 2) are also within the scope of the invention. The peptide fragments preferably have a CD100 activity.

A CD100 protein of the invention can be incorporated into a pharmaceutical composition which includes the protein (or active portion thereof) and a pharmaceutically acceptable carrier. In addition, vaccine compositions which include at least one antigen and a first agent which stimulates a CD100 ligand-associated signal in a cell, such as a leukocyte, e.g., a B cell or a T cell, are also within the scope of this invention. Such vaccine compositions can further include a second agent which stimulates a CD40-associated signal in a cell (e.g., a B cell).

The CD100 protein of the invention and agents which modulate a CD100 ligand-associated signal in a cell, such as a leukocyte can be used to modulate leukocyte responses *in vitro* or *in vivo*. In one embodiment, the invention provides a method for stimulating a CD100 ligand-associated signal in a B cell by contacting the cell with an agent that stimulates a CD100 ligand-associated signal. Such an agent can be, for example, a stimulatory form of a CD100 antigen (e.g., a soluble CD100 protein). Additional agents, such as an agent which provides a CD40 associated signal in the B cell can be used to stimulate a B cell response.

In another embodiment, a CD100 ligand-associated signal is inhibited to thereby inhibit a response by a B cell. In this embodiment, an agent which interacts with CD100, such as an anti-CD100 antibody can be used to inhibit a response by a B cell such as aggregation or differentiation. The methods of the invention for modulating B cell responses by manipulating the interaction of CD100 with its ligand can be applied *in vitro* (e.g., with cells in culture) or *in vivo*, wherein an agent that modulates CD100 and/or CD100 ligand is administered to the subject.

The invention further provides methods for modulating a T cell response comprising contacting a T cell with an agent which modulates a CD100 ligand-associated signal in the T cell. A T cell response is preferably T cell proliferation. The method also preferably includes contacting the T cell with a primary activation agent, such as an antibody to CD3 or at least one antigen.

Additional methods for modulating an interaction between immune cells and nerve cells in a subject by administering an amount of an agent which modulates a CD100 signal in the subject as well as methods for modulating and/or guiding axonal growth by contacting neurons with a modulating form of CD100, such that such that axonal growth is modulated and/or guided are also within the scope of the invention.

Brief Description of the Drawings

Figure 1 is the nucleotide sequence of a 4.2 kb cDNA encoding full length human CD100 (SEQ ID NO: 1) and the predicted amino acid sequence encoded by the cDNA (SEQ ID NO: 2).

Figure 2 is the predicted full length amino acid sequence of human CD100 (SEQ ID NO: 2) aligned for comparison to the semaphorin and Ig domains of Human semaphorin III (H-sema III) (SEQ ID NO: 3) and Mouse semaphorin C (M-Sema C) (SEQ ID NO: 4). The regions of identity are boxed. The putative hydrophobic region of the signal sequence is underlined; the sema domain is delimited by *; the gray box indicates an Ig-like domain; the transmembrane region is double-underlined; and the putative tyrosine phosphorylation site KPALTGY (SEQ ID NO: 5) (MAC Pattern: GenBank accession number PS00007) in the cytoplamic tail is boxed.

Figure 3 is a schematic representation of the structure of human CD100 indicating the different domains and the tyrosine phosphorylation site (Y).

Figure 4 represents a sequence comparison between the amino acid sequence of collapsin 4 (top sequence) and the amino acid sequence of a fragment of human CD100 (bottom sequence).

Figure 5 is a graphic representation of the result of flow cytometry analyses of COS cells transiently transfected with a CD100 cDNA (CD100), pCDM8 vector alone (Vector), and B7-1 cDNA (B7-1) and stained with the anti-CD100 antibodies BD16, F93G2, and BB18; and with the isotype matched B7-1 antibody B1.1.

Figure 6 are photographs depicting human splenic B cells cultured with mock transfected NIH-3T3 cells (t-mock) (Figure 6a); CD40L transfected NIH-3T3 cells (t-CD40L; Figures 6b and e) ; 50% mock transfected and 50% CD40L transfected NIH-3T3 cells (t-mock/t-CD40L) (Figue 6c), CD100 transfected NIH-3T3 cells (t-CD100) (Figure 6d); and 50% CD100 transfected and 50% CD40L transfected NIH-3T3 cells (t-CD100/t-CD40L) (Figure 6f), showing that CD100 stimulates B cell homotypic aggregation and that this effect synergizes with CD40 stimulation.

Figure 7 depicts a histogram showing the number of viable human B cells (x 10^6) after incubation of 10^6 B cells for 72 hours with mock transfected NIH-3T3 cells (t-mock); CD100 transfected NIH-3T3 cells (t-CD100); CD40 transfected NIH-3T3 cells (t-CD40); 50% mock transfected and 50% CD40L transfected NIH-3T3 cells (t-mock/t-CD40L); and 50% CD100 transfected and 50% CD40L transfected NIH-3T3 cells indicating that CD100

and CD40L stimulation prolong B cell survival and that CD100 and CD40L synergistically stimulate B cell survival.

Figure 8 is a graphic representation of a two color FACS analysis performed with anti-CD19 and anti-CD23 antibodies of human splenic B cells performed after 72 hours of co-culture with t-mock/t-CD40L cells or t-CD100/t-CD40L showing that CD100 reduces expression of CD40L-induced CD23 expression.

Figure 9 depicts the results of proliferation assays of CD4+ T cells incubated in media alone, with an anti-CD3 antibody, with an anti-CD3 antibody and NIH-3T3 cells transfected with a B7-1 cDNA (t-B7-1), with an anti-CD3 antibody and NIH-3T3 cells transfected with a B7-2 cDNA (t-B7-2), and with an anti-CD3 antibody and NIH-3T3 cells transfected with a CD100 cDNA (t-CD100) showing that CD100 is a T cell costimulatory molecule.

Figure 10 depicts the results of proliferation assays of CD4+ T cells incubated with an anti-CD3 antibody and 2 x 10^4 t-B7-1 cells and 0, 1 x 10^4, 2 x 10^4, or 3 x 10^4 t-B7-1 or t-CD100 cells.

Figure 11 depicts the results of proliferation assays of CD4+ T cells incubated with an anti-CD3 antibody and 2 x 10^4 t-B7-2 cells and 0, 1 x 10^4, 2 x 10^4, or 3 x 10^4 t-B7-2 or t-CD100 cells.

Figure 12 is the nucleotide sequence of a cDNA encoding murine CD100 (SEQ ID NO: 8).

Figure 13 shows a sequence comparison between the nucleic acid sequence of the open reading frame of human CD100 (bottom sequence, termed cd100, SEQ ID NO: 22 and nucleotides 88-2673 of SEQ ID NO: 1) and mouse CD100 (top sequence, termed mod2c-106, nucleotides 434-3016 of SEQ ID NO: 8). Hyphens indicate identical nucleotides and dots indicate gaps, i.e., nucleotides absent from one of the two sequences.

Figure 14 is the amino acid sequence of mouse CD100 (SEQ ID NO: 9).

Figure 15 shows an alignment of the amino acid sequences of portions of mouse and human CD100 proteins: a portion comprising the signal peptide domain (SEQ ID NO: 10 and SEQ ID NO: 11, respectively); a portion comprising the sema domain (SEQ ID NO: 12 and SEQ ID NO: 13, respectively); a portion comprising the Ig-like domain (SEQ ID NO: 14 and SEQ ID NO: 15, respectively); a portion comprising the stalk domain (SEQ ID NO: 16 and SEQ ID NO: 17, respectively); a portion comprising the transmembrane domain (SEQ ID NO: 18 and SEQ ID NO: 19, respectively); and a portion comprising the cytoplasmic domain (SEQ ID NO: 20 and SEQ ID NO: 21, respectively). Bars indicate identical amino acids and stars indicate conservative amino acid substitutions.

Detailed Description of the Invention

The invention pertains to novel CD100 antigens, or active portion thereof which are capable of stimulating a leukocyte response, such as a B cell response. The B cell response can be B cell aggregation. These CD100 antigens are preferably capable of inducing differentiation of B cells and/or are capable of increasing the viability of B cells and, thus, are involved in germinal center development (e.g., affinity maturation of antibodies). Even more preferred CD100 antigens are capable of stimulating a T cell response, such as T cell proliferation. A particularly preferred CD100 antigen is a human CD100 antigen which is expressed on various hematopoietic cells including activated T and B cells. The CD100 antigens of the invention are capable of synergizing with a CD40 ligand (e.g., gp39) to enhance stimulation of homotypic B cell aggregation and cell viability. Thus, the CD100 antigen amplifies a response of B cells to CD40. CD100 also modifies the response of B cells to CD40 by reducing the level of expression of CD23, a cell surface protein which is upregulated in response to stimulation with a CD40 ligand.

CD100 protein is a homodimeric transmembrane protein consisting of two polypeptide chains of about 150 kDa. The amino acid sequence of a polypeptide chain of human CD100 is shown in Figure 2 (SEQ ID NO: 2). The amino acid sequence of a polypeptide chain of mouse CD100 is shown in Figure 14 (SEQ ID NO: 9). As used herein, the term "CD100" is intended to include the homodimeric protein as well as a single CD100 polypeptide chain.

Each polypeptide chain of CD100 consists of several domains, shown schematically in Figure 3, which consist essentially of a signal peptide domain, a semaphorin domain, an immunoglobulin-like (Ig-like) domain, a stalk domain, a transmembrane domain, and a cytoplasmic domain. These domains are further described below.

(a) Signal peptide domain

As defined in the art, a signal peptide domain typically comprises of a hydrophobic domain and has small amino acids (e.g., glycine and alanine) located at amino acid positions -1 and -3 (amino acid position 1 being defined as the first amino acid of the mature form of the protein). A hydrophobic domain of human CD100 is located at about amino acids 9 to about amino acid 20. Small amino acids can be found at positions 19 (Glycine), 21 (Alanine), and 23 (Alanine) of human CD100 (Figures 1 and 2). Accordingly, a putative signal peptide cleavage site is located between amino acids 21 (Alanine) and 22 (Methionine) of human CD100. A preferred putative signal peptide cleavage site is located between amino acids 23 (Alanine) and 24 (Phenylalanine) of human CD100. Thus, cleavage at the first putative signal peptide cleavage site would result in a mature human CD100 protein having amino acids 22 to 262 of SEQ ID NO: 2. Cleavage at the second putative signal peptide cleavage site would result in a mature human CD100 protein having amino acids 24 to 262 of SEQ ID NO: 2. Similarly, in mouse CD100, a first putative signal peptide cleavage site is located between amino acids 21 (Alanine) and 22

(Valine). Cleavage at this first putative signal peptide cleavage site would result in a mature murine CD100 protein having amino acids 22 to 261 of SEQ ID NO: 9. A second putative signal peptide cleavage site in mouse CD100 is located between amino acids 23 (Alanine) and 24 (Phenylalanine). Cleavage at this second putative signal peptide cleavage site would result in a mature murine CD100 protein having amino acids 24 to 261 of SEQ ID NO: 9.

Various techniques can be used to identify which of the putative signal peptide cleavage sites (among those identified above and others) is in fact the site of cleavage of the mature CD100 protein in vivo. For, example, a CD100 protein on a cell surface can be isolated and the amino acid sequence of the N-terminus can be determined. Isolation of the CD100 protein must be done in conditions minimizing protein degradation by proteolytic enzymes.

(b) Semaphorin domain

As defined in the art, a semaphorin (sema) domain is an extracellular domain of approximately 500 amino acid containing 14-16 cysteines found specifically in proteins belonging to the semaphorin protein family. Accordingly, the sema domain of human and mouse CD100 can be defined as including about amino acid 42 to about amino acid 553 of SEQ ID NO: 2 and SEQ ID NO: 9, respectively. However, additional amino acids flanking amino acids 42 to 553 and which have significant homology to the sema domain of semaphorin family members can also be part of the semphorin domain. Similarly, the sema domain can also be defined as consisting of less than amino acids 42 to 553. All these fragments of CD100 are within the scope of the invention and intended to be included in the term "CD100 sema domain". A "CD100 sema domain includes about amino acids 42 to 553 of SEQ ID NO: 2 or SEQ ID NO: 9" is also intended to include portions of CD100 which can be defined as sema domains.

The phylogenetically conserved semaphorins have been characterized in insect, chicken, mouse and human nervous systems (Kolodkin A.L., et al., (1992), *Neuron* 9, 831; Luo Y. et al, (1993), *Cell* 75, 217; Kolodkin A. et al, (1993), *Cell* 75, 1389; Puschel, A. W., et al. (1995) *Neuron* 14:941; Messersmith, E.K et al. (1995) *Neuron* 14:949; published PCT patent application number PCT/US94/10151). Proteins having a semaphorin domain have also been found in vaccinia virus, variola virus (Kolodkin A. et al, (1993), *Cell* 75, 1389), and herpes virus (Ensser, A. and Fleckenstein, B.(1995) *J. Gen. Virol.* 76: 1063). However the viral semaphorins are only distantly related to the previously identified mammalian and insect semaphorins.

As shown in Figure 2, human CD100 shares certain structural features with H-Sema III and mouse Sema C (M-Sema C). H-Sema III and M-Sema C molecules contain the highly conserved 500 amino acid (aa) semaphorin (sema) domain followed by a C-2 type immunoglobulin-like (Ig) domain. Human CD100 shares 39% identity with H-sema III in

the sema domain and 33% identity in the Ig-like domain, whereas the rest of the protein is strikingly divergent. Human CD100 contains 15 of the 16 conserved sema domain cysteines and 9 putative N-linked glycoslyation sites. However, CD100 differs from H-Sema III and M-Sema C in that CD100 is a membrane bound protein, whereas H-Sema III and M-Sema C are secreted proteins. Other semaphorins related to collapsin have been isolated from chicken brains (Luo et al. (1995) *Neuron* 14:1131) and partially characterized. The partial amino acid sequence of one of these proteins, collapsin 4 (SEQ ID NO: 6), shares about 78% amino acid identity with a 295 amino acid long C-terminal portion of the human CD100 sema domain (amino acid residues 228-522 of the sequence shown in Figure 2 (SEQ ID NO: 2). The sequence comparison is shown in Figure 4. It is unknown whether collapsin 4 is a secreted protein or a membrane bound protein and whether it contains an Ig-like domain.

The human CD100 antigen is the first membrane bound protein shown to have a semaphorin domain and an Ig-like domain. Furthermore, human CD100 is the only membrane bound semaphorin having a tyrosine phosphorylation site in the cytoplasmic domain (see below). Based on its structure, CD100 can be defined as being a class IV semaphorin.

Accordingly, preferred CD100 antigens within the scope of the invention include a semaphorin domain or active portion thereof. Some members of the Semaphorin family have been shown to function as secreted or transmembrane proteins growth cone guidance molecules which are critical for guiding axonal growth of neurons. Membrane bound semaphorins include the grasshopper G-Sema I (also termed G-Fas IV), the Tribolium T-Sema I and the Drosophila D-Sema I. These proteins have an additional stretch of about 80 amino acids in the extracellular domain, a transmembrane domain, and an 80 to 110 amino acid cytoplasmic domain. Secreted semaphorins include the Drosophila D-Sema II, the chicken Collapsin, the human H-Sema III, and the mouse Sema C proteins. Secreted semaphorins have an additional stretch of about 20 amino acids in the extracellular domain, a single immunoglobulin domain and a 70-120 amino acid C-terminal region.

At least some semaphorins are involved in the nervous system development. Neurons secreting semaphorins such as chicken collapsin or its homolog human Sema III (H-sema III), selectively induce extending axonal growth processes to collapse and/or turn away and, therefore, not establish connections with the secreting neuron (Fan J. et al, (1995), *Neuron* 14, 263). The net effect of this collapse is a chemorepulsion and redirection of the extending processes. In contrast, human CD100 is the only semaphorin identified that functions in the hematopoietic system, in particular in modulating a B cell response.

The structural similarities between CD100 and the semaphorin family of proteins indicate that CD100 plays a role in the nervous system in addition to its role in the immune system. CD100 agents of the invention can also be used for modulating communication or

interaction between a neuron and an immune cell, such as a neuron. As such, the CD100 antigens of the invention will be used in modulating nerve cell development and/or modulating the interaction between the immune and nervous system.

(c) Immunoglobulin-like domain

As defined in the art, an immunoglobulin-like domain has certain characteristics and encompasses two cysteines separated by about 60 amino acids and including up to about 20 amino acids flanking this region on either side. Human and murine CD100 have a first cysteine located at amino acid position 576 and a second cysteine located at amino acid position 624 of amino acid sequences shown in Figure 1 (SEQ ID NO: 2) and in Figure 14 (SEQ ID NO: 9). Accordingly, a preferred immunoglobulin domain of CD100 corresponds to about amino acid 566 to about amino acid 630 of SEQ ID NO: 2 or SEQ ID NO: 9. However, additional flanking amino acids of amino acids 566-630 can also be included in the Ig-like domain. Accordingly, in one embodiment, the Ig-like domain of CD100 corresponds to about amino acids 554 to about amino acid 630. In another embodiment, an Ig-like domain can correspond to less than amino acids 566-630. These Ig-like domains are within the scope of the invention and are intended to be included in the term "CD100 Ig-like domain". A "CD100 Ig-like domain including amino acids 566-630 of SEQ ID NO: 2 or SEQ ID NO: 9" is also intended to include portions of CD100 which can be defined as Ig-like domains.

(d) Stalk domain

The stalk domain of CD100 is defined as a region located between the Ig-like domain and a transmembrane region. This region is rich in charged amino acids and has proteolytic enzyme recognition sites. Accordingly, the stalk domain corresponds to about amino acid 631 to about amino acid 733 of human CD100 (SEQ ID NO: 2) and to about amino acid 631 to about amino acid 732 of murine CD100 (SEQ ID NO: 9). The stalk domain can also include additional amino acid residues at the N-terminal end (e.g., residues which comprise the TM membrane). Alternatively, the stalk domain can include less residues than amino acids 631 to 732 or 733. These stalk domains are intended to be included in the term "CD100 stalk domain". The term "CD100 stalk domain including amino acids 631-733 of human CD100 (SEQ ID NO: 2) or amino acids 631-732 of murine CD100 (SEQ ID NO: 9)" is also intended to include portions of CD100 which can be defined as stalk domains.

The stalk domain allows a membrane bound CD100 protein to become soluble CD100 protein by proteolytic cleavage in this domain. In fact, soluble CD100 protein has been observed in supernatant from cells transfected with a CD100 cDNA. Accordingly, the CD100 nucleic acid of the invention allows production of membrane bound as well a soluble CD100 protein. Accordingly, within the scope of the invention are soluble CD100

proteins which are produced by proteolytic cleavage at the stalk region. Such proteins have similar uses as membrane bound CD100. Within the scope of the invention are methods for increasing production of soluble CD100, comprising incubating cells having membrane bound CD100 with a protease capable of cleaving the protein at the stalk region. Proteases capable of cleaving CD100 at the stalk domain can be identified according to the amino acid sequence of the stalk domain and other methods known in the art. Furthermore, the nucleic acid encoding the stalk domain can be used to produce recombinant proteins, e.g., other than CD100 proteins which will be membrane bound, but will be able to be cleaved from the membrane. It may be preferable to include a CD100 transmembrane domain in the recombinant construct containing the CD100 stalk domain. Accordingly, the invention provides nucleic acids comprising the stalk domain and the transmembrane domain of a CD100 protein, for, e.g., production of recombinant proteins which will be partly membrane bound and partly in solution.

(e) Transmembrane domain

As defined in the art, a transmembrane domain is a hydrophobic region. Accordingly, the transmembrane domain of human CD100 can be defined as a region from about amino acid 735 to about amino acid 753 of SEQ ID NO: 2 (Figures 1 and 2). The transmembrane domain of murine CD100 can be defined as a region from about amino acid 734 to about amino acid 752 of SEQ ID NO: 9. Also included in the term "CD100 transmembrane domain" are regions of CD100 corresponding essentially to amino acids 735-753 or 734-752, which lack one or more amino acids or which have one or more additional amino acids flanking this region and derived from the sequence of CD100.

Within the scope of the invention are nucleic acids encoding the transmembrane domain of CD100, which can be used to produce membrane bound recombinant proteins, e.g., proteins other than CD100. Accordingly, a nucleic acid encoding the transmembrane domain of CD100 can be genetically linked to a nucleic acid encoding a protein, such that the protein will be membrane bound.

(f) Cytoplasmic domain

The cytoplasmic domain of CD100 can be defined as the C-terminal portion of the protein following the transmembrane domain. Accordingly, a cytoplasmic domain of human CD100 is a portion of the protein from about amino acid 754 to about amino acid 862 of SEQ ID NO: 2. A cytoplasmic domain of murine CD100 is a portion of the protein from about amino acid 753 to about amino acid 261 of SEQ ID NO: 9.

A consensus site for tyrosine phosphorylation, KPALTGY (SEQ ID NO: 5) from amino acid 808 to amino acid 813 in the cytoplasmic tail of human CD100 and from amino acid 807 to amino acid 812 of murine CD100 supports the predicted association of CD100 with a tyrosine kinase (Sidorenko S., et al. "Identification of antigens associated with

protein kinases by activation antigens panel mAb." S. F. Schlossman, et al., Eds., *Leucocyte Typing V* (Oxford University Press, Oxford, 1995), vol. 1.; RuddC., et al., "Identification of antigens associated with protein kinases by activation antigen panel mAb." S. F. Schlossman, et al., Eds., *Leucocyte Typing V* (Oxford University Press, Oxford, 1995), vol. 1. Accordingly, the tyrosine residue of this tyrosine phosphorylation site can be phosphorylated.

The cytoplasmic domain of CD100 can be used, e.g.. for identifying proteins or other molecules which interact with the cytoplasmic domain and which are, e.g., involved in signal transduction.

A CD100 molecule of the invention can thus be defined as comprising a signal sequence of about 21 or 23 amino acids (about amino acids 1-21 or about amino acids 1-23 of the sequence shown in Figure 2 (SEQ ID NO: 2) or the sequence shown in Figure 14 (SEQ ID NO: 9)) followed by a semaphorin domain of about 512 amino acids (about amino acids 42-553 of the sequence shown in Figure 2 (SEQ ID NO: 2) or the sequence shown in Figure 14 (SEQ ID NO: 9)), an immunoglobulin-like (Ig-like) domain of about 65 amino acids (about amino acids 566-630 of the sequence shown in Figure 2 (SEQ ID NO: 2) or the sequence shown in Figure 14 (SEQ ID NO: 9)), a lysine-rich stretch of 104 amino acids, a stalk domain of about 103 or 104 amino acids (about amino acids 631-734 of the sequence shown in Figure 2 (SEQ ID NO: 2) or amino acids 631-733 of the sequence shown in Figure 14 (SEQ ID NO: 9)), a hydrophobic transmembrane region of about 19 amino acids (about amino acids 735-753 of the sequence shown in Figure 2 (SEQ ID NO: 2) or about amino acids 734-752 of the sequence shown in Figure 14 (SEQ ID NO: 9)), and a cytoplasmic tail of about 109 amino acids (about amino acids 754-862 of the sequence shown in Figure 2 (SEQ ID NO: 2) or about amino acids 753-861 of the sequence shown in Figure 14 (SEQ ID NO: 9)).

Accordingly, this invention pertains to CD100 molecules (e.g., antigens, proteins) and to active portions thereof, such as peptides having an activity of CD100. The phrases "an activity of CD100" or "having a CD100 activity" are used interchangeably herein to refer to molecules such as proteins and peptides which are capable of stimulating a leukocyte response, such as a B cell response, e.g, B cell aggregation, and which preferably include a semaphorin domain or an active portion thereof. The phrase "active portion of a semaphorin domain" refers to those portions of a semaphorin domain that are required to mediate, alone, or in collaboration with other portions of the protein, a CD100 activity. Preferred molecules having a CD100 activity are those which are capable of stimulating B cell differentiation. Other preferred molecules having a CD100 activity are capable of stimulating B cell viability, and/or reducing the expression of CD23. Molecules having a CD100 activity are also intended to include molecules which are capable of stimulating a T cell response, such as stimulating proliferation of T cells in the presence of a primary activation signal and which preferably include a s maphorin domain or active portion

thereof. Even more preferred molecules of the invention include CD100 antigens which are capable of stimulating a B cell response and a T cell response. Particularly preferred molecules within the scope of the invention include those which are structurally similar to human CD100, i.e., molecules which preferably comprise in addition to the sema domain, an immunoglobulin-like domain, a transmembrane domain, a cytoplasmic domain having a tyrosine phosphorylation site, or any combination of these domains.

Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic acid Molecules

One aspect of this invention pertains to isolated nucleic acid molecules that encode a novel CD100 antigen, such as human CD100, fragments of such nucleic acids, or equivalents thereof. The term "nucleic acid molecule" as used herein is intended to include such fragments or equivalents and refers to DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA). The nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA. An "isolated" nucleic acid molecule is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, may be free of other cellular material.

The term "equivalent" is intended to include nucleotide sequences encoding functionally equivalent CD100 antigens or functionally equivalent peptides having a CD100 activity. Functionally equivalent CD100 antigens or peptides is intended to include antigens which are capable of stimulating a leukocyte response, such as a B cell response, e.g., B cell aggregation and preferably contain a semaphorin domain or an active portion thereof. Functionally equivalent CD100 antigens are also preferably capable of stimulating B cell differentiation, prolonging B cell viability, and/or decreasing CD23 expression. Functionally equivalent CD100 antigens or peptides also include antigens which are capable of providing a costimulatory signal to T cells and preferably contain a semaphorin domain or an active portion thereof. Preferred functionally equivalent CD100 antigens include antigens which are capable of stimulating a B cell response and a T cell response, and which preferably comprise a semaphorin domain or active portion thereof.

Other equivalents of CD100 antigens include structural equivalents. Structural equivalents of a CD100 antigen preferably comprise a semaphorin domain or portion thereof, an Ig-like domain or portion thereof, a transmembrane domain or portion thereof, and/or a cytoplasmic domain or portion thereof. A portion of a CD100 antigen is at least about 20 amino acid residues in length, more preferably at least about 40 amino acid residues in length, and even more preferably at least about 60 amino acid r sidues in length.

Preferred nucleic acids of the invention include nucleic acid molecules comprising a nucleotide sequence provided in Figure 1 (SEQ ID NO: 1) or Figure 12 (SEQ ID NO: 8), fragments thereof or equivalents thereof.

In one embodiment, the invention pertains to a nucleic acid molecule which is a naturally occuring form of a nucleic acid molecule encoding a CD100 antigen, such as a CD100 antigen having an amino acid sequence shown in Figure 2 (SEQ ID NO: 2) or in Figure 14 (SEQ ID NO: 9). Preferably, a naturally occuring form of a nucleic acid encoding CD100 is derived from hematopoietic cells, such as activated lymphocytes. Such naturally occuring equivalents can be obtained, for example, by sceening a cDNA library, prepared with RNA from hematopoietic cells, with a nucleic acid molecule having a sequence shown in Figure 1 (SEQ ID NO: 1) or in Figure 12 (SEQ ID NO: 8) under high stringency hybridization conditions. Such conditions are further described herein.

Also within the scope of the invention are nucleic acids encoding natural variants and isoforms of CD100 antigens, such as splice forms. Northern blot hybridization of a CD100 cDNA to RNA from lymphocytes and non-hematopoietic tissues indicated the existence of several mRNA hybridizing to CD100 DNA (Example 2). Such natural variants are within the scope of the invention. Furthermore, it is likely that secreted isoforms of CD100 exit, since CD100 is related to proteins from the semaphorin family, which are either secreted or membrane bound proteins. Such naturally secreted forms of CD100 are also within the scope of the invention.

In a preferred embodiment, the nucleic acid molecule encoding a CD100 antigen is a cDNA. Preferably, the nucleic acid molecule is a cDNA molecule consisting of at least a portion of a nucleotide sequence encoding human CD100, as shown in Figure 1 (SEQ ID NO: 1). A preferred portion of the cDNA molecule of Figure 1 (SEQ ID NO: 1) includes the coding region of the molecule (SEQ ID NO: 22). Other preferred portions include those which code for domains of CD100, such as the extracellular domain, the semaphorin domain, the immunoglobulin domain, the transmembrane domain, the cytoplasmic domain, or any combination thereof.

In another embodiment, the nucleic acid of the invention encodes a CD100 antigen or active fragment thereof having an amino acid sequence shown in Figure 2 (SEQ ID NO: 2) or in Figure 14 (SEQ ID NO: 9). In yet other embodiment, preferred nucleic acid molecules encode a peptide having an overall amino acid sequence identity or similarity of at least about 50%, more preferably at least about 60%, more preferably at least about 70 %, more preferably at least about 80%, more preferably 86% and most preferably at least about 90% with an amino acid sequence shown in Figure 2 (SEQ ID NO: 2) or in Figure 14 (SEQ ID NO: 9). Preferred nucleic acids within the scope of the invention include nucleic acids encoding a protein or peptide having an overall similarity of at least about 91% with an amino acid sequence SEQ ID NO: 2. Nucleic acids which encode peptides having an overall amino acid sequence identity or similarity of at least about 93%, more preferably at

least about 95%, and most preferably at least about 98-99% with a sequence set forth in Figure 2 (SEQ ID NO: 2) are also within the scope of the invention. Homology, also termed herein "identity" refers to sequence similarity between two protein (peptides) or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequences is occupied by the same nucleotide base or amino acid, then the molecules are homologous, or identical, at that position. A degree (or percentage) of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. When a position in the compared amino acid sequences is occupied by an amino acid of a similar type, i.e., a conserved amino acid (see definition herein) then the molecules are similar in that position. A degree of similarity between amino acid sequences is defined as the proportion of the sum of the number of identical and conserved amino acid, and the total number of amino acids.

Isolated nucleic acids encoding a peptide having a CD100 activity, as described herein, and having a sequence which differs from nucleotide sequence shown in Figure 1 (SEQ ID NO: 1) or Figure 12 (SEQ ID NO: 8) due to degeneracy in the genetic code are also within the scope of the invention. Such nucleic acids encode functionally equivalent peptides (e.g., having a CD100 activity) or structurally equivalent peptides but differ in sequence from the sequence of Figure 2 (SEQ ID NO: 2) or Figure 14 (SEQ ID NO: 9) due to degeneracy in the genetic code. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may occur due to degeneracy in the genetic code. As one example, DNA sequence polymorphisms within the nucleotide sequence of a CD100 antigen (especially those within the third base of a codon) may result in "silent" mutations in the DNA which do not affect the amino acid encoded. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the CD100 antigen will exist within a population. It will be appreciated by one skilled in the art that these variations in one or more nucleotides (up to about 3-4% of the nucleotides) of the nucleic acids encoding peptides having the activity of a CD100 antigen may exist among individuals within a population due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of the invention. Furthermore, there may be one or more isoforms or related, cross-reacting family members of the CD100 antigen described herein. Such isoforms or family members are defined as proteins related in function and amino acid sequence to a CD100 antigen, but encoded by genes at different loci. Such isoforms or family members are within the scope of the invention.

A "fragment" of a nucleic acid encoding a CD100 antigen is defined as a nucleotide sequence having fewer nucleotides than the nucleotide sequence encoding the entire amino acid sequence of a CD100 antigen, such as human CD100. A fragment of a nucleic acid is

at least about 20 nucleotides, preferably at least about 30 nucleotides, more preferably at least about 40 nucleotides, even more preferably at least about 50 nucleotides long. Also within the scope of the invention are nucleic acid fragments which are at least about 60, 70, 80, 90, 100 or more nucleotides long. Preferred fragments include fragments which encode a peptide having a CD100 activity (i.e., the ability to stimulate a leukocyte response, such as a B cell response, e.g., B cell aggregation, or a T cell response, e.g., T cell proliferation in the presence of a primary activation signal and which preferably contain a sema domain or an active portion thereof). Thus, a preferred peptide having a CD100 activity has a sema domain or active portion thereof, and stimulates aggregation of B cells. Another preferred peptide having CD100 activity has a sema domain or active portion thereof and provides a costimulatory signal to T cells. For determining whether a fragment of a CD100 antigen, such as a fragment of human CD100 is capable of inducing a B cell response, such as B cell aggregation, the peptide, either in soluble form of membrane bound is added to a culture of B cells. Aggregation of B cells can then be visualized macroscopically or under a microscope. Preferred conditions for a B cell aggregation assay are described in the Example section. Assays for determining whether a fragment of a CD100 antigen is capable of providing a costimulatory signal to T cells the following assay can be performed. T cells, such as CD4+ T cells, are incubated in the presence of a primary activation signal, such as an anti-CD3 antibody and various amounts of a CD100 fragment, e.g., expressed on the surface of cells. Following incubation for about 3 days, a proliferation assay is performed, which is indicative of the proliferation rate of the T cells. Thus, a fragment of a CD100 antigen which is capable of costimulating T cells is a fragment of a CD100 antigen which in the presence of a primary T cell activation signal stimulates the T cells to proliferate at a rate that is greater than proliferation rate of T cells contacted only with a primary activation signal. Proliferation assays can also be performed as described in the PCT Application No. PCT/US94/08423.

The fragments of a CD100 antigen, such as human CD100, are also preferably capable of stimulating B cell differentiation. For example, a CD100 fragment of the invention can stimulate the differentiation of B cells from a lymphoblast to a centrocyte. Other CD100 fragments of the invention stimulate the differentiation of B cells into antibody secreting plasma cells or memory B cells. It is also possible that the same fragment will stimulate differentiation of B cells into either memory B cells or antibody secreting B cells depending on the conditions of stimulation. Increased B cell differentiation can be shown, for example, by comparing the expression of B cell markers on B cells incubated in the presence or absence of the CD100 protein fragments. B cells at specific stages of differentiation are characterized by the expression of specific membrane proteins. For example, CD23, CD38, and CD40 are expressed on activated B cells. However, centrocytes and plasma cells lose membrane expression of CD23, CD38 and immunoglobulin molecules, such as IgM and IgD. A plasma cell can also be identified by

its secretion of antiboby. Thus, the ability of a peptide of the invention to induce B cell differentiation can be shown by measuring surface levels of specific B cell markers. Preferred fragments of the invention are capable of reducing the level of expression of CD23 induced by CD40 crosslinking. This can be demonstrated, for example, by CD23 fluorescence staining and flow cytometry analysis of B cells stimulated through CD40 and incubated in the presence or absence of CD100 fragments. Methods that can be used for showing the biological activities of CD100 on B cells are further described in the Example section.

Other preferred nucleic acid fragments encode peptides comprising at least part of one or more specific domains of a CD100 antigen. Preferred nucleic acid fragments encode peptides of at least about 20 amino acid residues in length, preferably at least about 40 amino acid residues and length, and more preferably at least about 60 amino acid residues in length. Nucleic acid fragments which encode peptides of at least about 80 amino acid residues in length, at least about 100 amino acid residues in length, at least about 200 amino acid residues in length, at least about 300 amino acid residues in length, at least about 400 amino acid residues in length, at least about 500 amino acid residues in length, or more amino acids in length are also within the scope of the invention. Particularly preferred nucleic acid fragments encode a peptide having an amino acid sequence represented by a formula:

$$X_n\text{-}Y\text{-}Z_m$$

In the formula, Y comprises the semaphorin domain. Y is preferably a peptide having about amino acid residues 42-553 of the sequence shown in Figure 2 (SEQ ID NO: 2). Y can also be a peptide having about amino acid residues 42-553 of the sequence shown in Figure 14 (SEQ ID NO: 9). X_n and Z_m are additional amino acid residue(s) linked to Y by an amide bond. X_n and Z_m are selected from amino acid residues contiguous to Y in the amino acid sequence shown in Figure 2 (SEQ ID NO: 2) or Figure 14 (SEQ ID NO: 9). In the formula, X_n is amino acid residue(s) selected from amino acids contiguous to the amino terminus of Y in the sequence shown in Figure 2 (SEQ ID NO: 2) or Figure 14 (SEQ ID NO: 9), i.e., from amino acid residue 41 to 1. Z_m is amino acid residue(s) selected from amino acids contiguous to the carboxy terminus of Y in the sequence shown in Figure 2 (SEQ ID NO: 2) or Figure 14 (SEQ ID NO: 9), i.e., from amino acid residue 554 to 862. In addition, in the formula, n is a number from 0 to 41 (n=0-42) and m is a number from 0 to 310 (m=0-310). A particularly preferred peptide has an amino acid sequence represented by the formula X_n-Y-Z_m as above, where n=0 and m=0.

Particularly preferred nucleic acid fragments encode a peptide having an amino acid sequence represented by a formula X_n-Y-Z_m, wherein Y is selected from the group consisting of: an extracellular domain, which is preferably about amino acid residues 22-

630, amino acid residues 24-630, amino acid residues 22-734, or amino acid residues 24-734 of the sequence shown in Figure 2 (SEQ ID NO: 2) or amino acid residues 22-630, amino acid residues 24-630, amino acid residues 22-733, or amino acid residues 24-733 of the sequence shown in Figure 14 (SEQ ID NO: 9); an extracellular domain comprising the signal peptide, which is preferably about amino acid residues 1-630 or 1-734 of the sequence shown in Figure 2 (SEQ ID NO: 2) or amino acid residues 1-630 or 1-733 of the sequence shown in Figure 14 (SEQ ID NO: 9); an Ig-like domain, which is preferably about amino acid residues 566-630 or 554-630 of the sequence shown in Figure 2 (SEQ ID NO: 2) or in Figure 14 (SEQ ID NO: 9); a transmembrane domain, which is about amino acid residues 735-753 of the sequence shown in Figure 2 (SEQ ID NO: 2) or in Figure 14 (SEQ ID NO: 9); and a cytoplasmic domain, which is about amino acid residues 754-862 of the sequence shown in Figure 2 (SEQ ID NO: 2) or in Figure 14 (SEQ ID NO: 9). In the formula, X_n and Z_m are additional amino acid residues linked to Y by an amide bond. X_n and Z_m are amino acid residues selected from amino acids contiguous to Y in the amino acid sequence shown in Figure 2 (SEQ ID NO: 2) or in Figure 14 (SEQ ID NO: 9). X_n is amino acid residues selected from amino acids contiguous to the amino terminus of Y in the sequence shown in Figure 2 (SEQ ID NO: 2) or in Figure 14 (SEQ ID NO: 9). Z_m is amino acid residues selected from amino acids contiguous to the carboxy terminus of Y in the sequence shown in Figure 2 (SEQ ID NO: 2) or in Figure 14 (SEQ ID NO: 9). According to the formula, n is a number from 0 to 753 (n=0-753) and m is a number from 0 to 234 (m=0-234). A particularly preferred peptide has an amino acid sequence represented by the formula X_n-Y-Z_m, where n=0 and m=0.

Nucleic acids of the invention also include nucleic acids encoding peptides having a formula X_n-Y-Z_m, wherein Y is a portion of any of the domains described above and X_n and Z_m are amino acids flanking Y.

Also within the scope of the invention are nucleic acids encoding a signal domain having at least about 68% identity with a signal domain of SEQ ID NO: 2 or SEQ ID NO: 9, nucleic acids encoding a sema domain having at least about 88% identity with a sema domain of SEQ ID NO: 2 or SEQ ID NO: 9, nucleic acids encoding an Ig-like domain having at least about 84% identity with an Ig-like domain of SEQ ID NO: 2 or SEQ ID NO: 9, nucleic acids encoding a stalk domain having at least about 70% identity with a stalk domain of SEQ ID NO: 2 or SEQ ID NO: 9, nucleic acids encoding a transmembrane domain having at least about 84% identity with a transmembrane domain of SEQ ID NO: 2 or SEQ ID NO: 9, and nucleic acids encoding a cytoplasmic domain having at least about 94% identity with a cytoplasmic domain of SEQ ID NO: 2 or SEQ ID NO: 9. Proteins encoded by the above-described nucleic acids are also within the scope of the invention.

Other nucleic acids within the scope of the invention include nucleic acids encoding a peptide having the formula X_n-Y-Z_m, wherein Y comprises an amino acid sequence that is at least about 50%, preferably at least about 60%, more preferably at least about 70%,

more preferably at least about 80%, more preferably at least about 90%, and most preferably at least about 95% identical in amino acid sequence with an amino acid sequence of the semaphorin domain, the extracellular domain, the Ig-like domain, or the cytoplasmic domain shown in Figure 2 (SEQ ID NO: 2). In yet other embodiment, the amino acid sequence represented by X_n and Z_m are at least about 50%, preferably at least about 60%, more preferably at least about 70%, more preferably at least about 80%, more preferably at least about 90%, and most preferably at least about 95% identical in amino acid sequence with an amino acid sequence shown in Figure 2, SEQ ID NO: 2.

Yet other preferred nucleic acids of the invention comprise several nucleic acids defined above. Thus, within the scope of the invention are nucleic acids encoding a peptide having a formula X_n-Y-Z_m, wherein Y comprises a semaphorin domain and a peptide having the same formula, wherein Y comprises an Ig-like domain. Further within the scope of the invention are nucleic acids encoding a peptide having a formula X_n-Y-Z_m, wherein Y comprises a semaphorin domain and a peptide having the same formula, wherein Y comprises a transmembrane domain.

The invention also pertains to nucleic acid molecules comprising a nucleic acid encoding a semaphorin domain, or an active portion thereof, that is at least about 60%, preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, even more preferably at least about 95%, and most preferably at least about 98% identical in amino acid sequence with the semaphorin domain of human CD100 shown in Figure 2 (SEQ ID NO: 2). Preferred nucleic acids comprise an open reading frame having at least about 83% identity with an open reading frame of SEQ ID NO: 1. Also within the scope of the invention are nucleic acids comprising a first nucleic acid encoding a semaphorin domain, or an active portion thereof, which is at least about 50%, preferably at least about 60%, more preferably at least about 70%, even more preferably at least about 80%, and even more preferably at least about 90% identical in amino acid residues to the semaphorin domain of the human CD100 shown in Figure 2 (SEQ ID NO: 2) and a second nucleic acid encoding an Ig-like domain, which is at least about 40%, preferably at least about 50%, more preferably at least about 60%, more preferably at least about 70%, more preferably at least about 80%, and most preferably at least about 90% identical in amino acid sequence with the Ig-like domain of the human CD100 shown in Figure 2 (SEQ ID NO: 2). Even more preferred nucleic acids comprise a third nucleic acid encoding a cytoplasmic domain, which is at least about 40%, preferably at least about 50%, more preferably at least about 60%, more preferably at least about 70%, more preferably at least about 80%, and most preferably at least about 90% identical in amino acid sequence with a cytoplasmic domain of the human CD100 shown in Figure 2 (SEQ ID NO: 2).

Nucleic acid fragments within the scope of the invention include those capable of hybridizing with nucleic acids from other animal species for use in screening protocols to detect novel proteins that are related to the CD100 antigen described herein. These and

other fragments are described in detail herein. Generally, the nucleic acid encoding a fragment of a CD100 antigen will be selected from the bases coding for the mature protein, however, in some instances it may be desirable to select all or part of a fragment or fragments from the leader or signal sequence or non-coding portion of a nucleotide sequence. Preferred nucleic acid fragments for detecting and/or isolating nucleic acids encoding a CD100 antigen comprise any portion of the coding sequence of CD100 excluding the semaphorin domain. In other embodiments, it may be desirable to select a nucleic acid encoding the semaphorin domain. Nucleic acids within the scope of the invention may also contain linker sequences, modified restriction endonuclease sites and other sequences useful for molecular cloning, expression or purification of recombinant protein or fragments thereof. These and other modifications of nucleic acid sequences are described in further detail herein.

A nucleic acid encoding a peptide having an activity of a CD100 activity, such as human CD100, can be obtained from mRNA present in activated B lymphocytes or activated T lymphocytes. Alternatively, a nucleic acid encoding a peptide having an activity of a CD100 antigen can be obtained from other cells and tissues, since CD100 mRNA was also observed in tissues such as skeletal muscle (Example2). It should also be possible to obtain nucleic acid sequences encoding such CD100 antigens from genomic DNA. For example, the gene encoding the CD100 antigen can be cloned from either a cDNA or a genomic library in accordance with protocols herein described. A cDNA encoding a CD100 antigen can be obtained by isolating total mRNA from an appropriate cell line. Double stranded cDNAs can then be prepared from the total mRNA. Subsequently, the cDNAs can be inserted into a suitable plasmid or viral (e.g., bacteriophage) vector using any one of a number of known techniques. Genes encoding CD100 antigens can also be cloned using established polymerase chain reaction techniques in accordance with the nucleotide sequence information provided by the invention. The nucleic acids of the invention can be DNA or RNA. A preferred nucleic acid is a cDNA encoding the human CD100 antigen having the sequence depicted in Figure 1 (SEQ ID NO: 1).

Another aspect of the invention provides a nucleic acid which hybridizes under high or low stringency conditions to a nucleic acid which encodes a peptide having all or a portion of an amino acid sequence shown in Figure 2 (SEQ ID NO: 2). Appropriate stringency conditions which promote DNA hybridization, for example, 6.0 X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 X SSC at 50°C are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 X SSC at 25 °C to a high stringency of about 0.2 X SSC at 65°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high

stringency conditions, at about 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of Figure 1, SEQ ID NO: 1 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein). In one embodiment, the nucleic acid encodes a natural CD100.

A cDNA encoding mouse CD100 has been cloned by cross-hybridization according to the methods described in the previous paragraph (Example 8). Accordingly, other nucleic acids can be obtained by similar methods.

In addition to naturally-occurring allelic variants of the CD100 sequence that may exist in the population, the skilled artisan will further appreciate that changes may be introduced by mutation into the nucleotide sequence of Figure 1, SEQ ID NO: 1 or Figure 12 (SEQ ID NO: 8), thereby leading to changes in the amino acid sequence of the encoded CD100 protein, without altering the functional ability of the CD100 protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues may be made in the sequence of Figure 1, SEQ ID NO: 1. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of CD100 (e.g., the sequence of Figure 2, SEQ ID NO: 2) without altering the ability of CD100 to stimulate a leukocyte response, such as a B cell response, i.e., B cell aggregation, whereas an "essential" amino acid residue is required for such activity. For example, amino acid residues of CD100 that are strongly conserved within members of a semaphorin family are predicted to be essential to CD100 activity and thus are not likely to be amenable to alteration.

An isolated nucleic acid molecule encoding a CD100 protein homologous to the protein of Figure 2, SEQ ID NO: 2 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of Figure 1, SEQ ID NO: 1 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into Figure 1, SEQ ID NO: 1 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in CD100 is preferably replaced with

another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a CD100 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for or CD100 activity to identify mutants that retain CD100 activity. Following mutagenesis of a nucleic acid having a sequence shown in Figure 1, SEQ ID NO: 1, the encoded protein can be expressed recombinantly and activity of the protein can be determined. A suitable assay for testing the activity of a CD100 protein or active portion thereof and mutated CD100 proteins is described in further detail herein.

In addition to the nucleic acid molecules encoding CD100 proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid.

The antisense nucleic acid can be complementary to an entire CD100 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding CD100. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (e.g., the entire coding region of Figure 1, SEQ ID NO: 1). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding CD100. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding CD100 disclosed herein (e.g., Figure 1, SEQ ID NO: 1), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule may be complementary to the entire coding region of CD100 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of CD100 mRNA. For example, the antisense oligonucleotide may be complementary to the region surrounding the translation start site of CD100 mRNA. An antisense oligonucleotide can be, for example, about 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Alternatively, the antisense

nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

In another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. A ribozyme having specificity for a CD100-encoding nucleic acid can be designed based upon the nucleotide sequence of a CD100 cDNA disclosed herein (i.e., SEQ ID NO: 1). See for example Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, CD100 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See for example Bartel, D. and Szostak, J.W. (1993) *Science* 261: 1411-1418.

The nucleic acid sequences of the invention can also be chemically synthesized using standard techniques. Various methods of chemically synthesizing polydeoxynucleotides are known, including solid-phase synthesis which, like peptide synthesis, has been fully automated in commercially available DNA synthesizers (See e.g., Itakura et al. U.S. Patent No. 4,598,049; Caruthers et al. U.S. Patent No. 4,458,066; and Itakura U.S. Patent Nos. 4,401,796 and 4,373,071, incorporated by reference herein).

II. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding CD100 (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors,

such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., CD100 proteins, mutant forms of CD100, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of CD100 in prokaryotic or eukaryotic cells. For example, CD100 can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector may be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promotors directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and th recombinant protein to enable separation of the recombinant protein from

the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann et al., (1988) *Gene* 69:301-315) and pET 11d (Studier et al., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al., (1992) *Nuc. Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the CD100 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerivisae* include pYepSec1 (Baldari. et al., (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA).

Alternatively, CD100 can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al., (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow, V.A., and Summers, M.D., (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B., (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987), *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

In one embodiment, a recombinant expression vector containing DNA encoding a CD100 fusion protein is produced. A CD100 fusion protein can be produced by recombinant expression of a nucleotide sequence encoding a first peptide having a CD100 activity and a nucleotide sequence encoding a second peptide corresponding to a moiety that alters a characterisic of the first peptide, e.g., its solubility, affinity, stability or valency, for example, an immunoglobulin constant region. Preferably, the first peptide consists of a portion of the extracellular domain of a CD100 antigen, such as the human CD100 antigen (e.g., approximately amino acid residues 22-734 of the sequence shown in Figure 2 (SEQ ID NO: 2)) or a semaphorin domain of a CD100 antigen, such as human CD100 (e.g., approximately amino acid residues 42-553 of the sequence shown in Figure 2 (SEQ ID NO: 2)). Preferred nucleic acids encoding a CD100Ig fusion protein comprise a nucleic acid encoding a peptide from the group of peptides having about amino acid residues 1-553, about amino acid residues 22-553, about amino acid residues 1-630, and about amino acid residues 22-630 of human CD100 shown in Figure 2 (SEQ ID NO: 2). The second peptide can include an immunoglobulin constant region, for example. a human C1 domain or C4 domain (e.g., the hinge, CH2 and CH3 regions of human IgC1, or human IgC4, see e.g., Capon et al. US 5,116,964, incorporated herein by reference). The immunoglobulin constant region may contain genetic modifications which reduce or eliminate effector activity inherent in the immunoglobulin structure. For example, DNA encoding the extracellular portion of human CD100 can be joined to DNA encoding the hinge, CH2 and CH3 regions of human IgC1 and/or IgC4 modified by site directed mutagenesis. A resulting CD100Ig fusion protein may have altered CD100 solubility, binding affinity, stability and/or valency (i.e., the number of binding sites available per molecule) and may

increase the efficiency of protein purification. Fusion proteins and peptides produced by recombinant techniques may be secreted and isolated from a mixture of cells and medium containing the protein or peptide. Alternatively, the protein or peptide may be retained cytoplasmically and the cells harvested, lysed and the protein isolated. A cell culture typically includes host cells, media and other byproducts. Suitable mediums for cell culture are well known in the art. Protein and peptides can be isolated from cell culture medium, host cells, or both using techniques known in the art for purifying proteins and peptides. Techniques for transfecting host cells and purifying proteins and peptides are described in further detail herein.

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to CD100 RNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to recombinant host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell may be any prokaryotic or eukaryotic cell. For example, CD100 protein may be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium

chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker may be introduced into a host cell on the same vector as that encoding CD100 or may be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

Transfected cells which express peptides having a CD100 activity on the surface of the cell are also within the scope of this invention. In one embodiment, a host cell such as a COS cell is transfected with an expression vector directing the expression of a peptide having a CD100 activity on the surface of the cell. Such a transfected host cell can be used in methods for identifying molecules which inhibit binding of CD100 to its receptor or ligand on, e.g., B cells or which interfere with intracellular signaling in response to CD100 interaction. Such cells can also be used for stimulating B cell aggregation and/or B cell differentiation and/or T cell proliferation according to the methods of the invention. In another embodiment, a tumor cell such as a sarcoma, a melanoma, a leukemia, a lymphoma, a carcinoma or a neuroblastoma is transfected with an expression vector directing the expression of at least one peptide having a CD100 activity on the surface of the tumor cell. In some instances, it may be beneficial to transfect a tumor cell to coexpress other molecules, including a CD40 ligand, major histocompatibility complex (MHC) proteins, for example MHC class II and chain proteins or an MHC class I chain protein, and, if necessary, a β_2 microglobulin protein. Such transfected tumor cells can be used to induce tumor immunity in a subject.

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) CD100 protein. Accordingly, the invention further provides methods for producing CD100 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding CD100 has been introduced) in a suitable medium until CD100 is produced. In another embodiment, the method further comprises isolating CD100 from the medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which CD100-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous CD100 sequences have been introduced into their genome or homologous recombinant animals in which endogenous CD100 sequences have been altered. Such animals are useful for studying the function and/or activity of CD100 and for identifying and/or evaluating modulators of CD100 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which one or more of the cells of the animal includes a transgene. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous CD100 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing CD100-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The human CD100 cDNA sequence of Figure 1, SEQ ID NO: 1 or the murine cDNA sequence shown in Figure 12, SEQ ID NO: 8 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human CD100 gene, such as a mouse CD100 gene, can be isolated based on hybridization to the human CD100 cDNA (described further in subsection I above) and used as a transgene. Such a mouse cDNA has been isolated (see Example 8 herein). Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the CD100 transgene to direct expression of CD100 protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Patent No. 4,873,191 by Wagner et al. and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the CD100 transgene in its genome and/or expression of CD100 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional

animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding CD100 can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a CD100 gene into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the CD100 gene. The CD100 gene may be a human gene (e.g., from a human genomic clone isolated from a human genomic library screened with the cDNA of Figure 1, SEQ ID NO: 1), but more preferably, is a non-human homologue of a human CD100 gene. For example, a mouse CD100 gene can be isolated from a mouse genomic DNA library using the human CD100 cDNA of Figure 1, SEQ ID NO: 1 or preferably the murine cDNA of Figure 12, SEQ ID NO: 8 as a probe. The mouse CD100 gene then can be used to construct a homologous recombination vector suitable for altering an endogenous CD100 gene in the mouse genome. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous CD100 gene is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous CD100 gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous CD100 protein). In the homologous recombination vector, the altered portion of the CD100 gene is flanked at its 5' and 3' ends by additional nucleic acid of the CD100 gene to allow for homologous recombination to occur between the exogenous CD100 gene carried by the vector and an endogenous CD100 gene in an embryonic stem cell. The additional flanking CD100 nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see e.g., Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced CD100 gene has homologously recombined with the endogenous CD100 gene are selected (see e.g., Li, E. et al. (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see e.g., Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International

Publication Nos.: WO 90/11354 by Le Mouellec et al.; WO 91/01140 by Smithies et al.; WO 92/0968 by Zijlstra et al.; and WO 93/04169 by Berns et al.

III. Isolated CD100 Proteins and Anti-CD100 Antibodies

Another aspect of the invention pertains to isolated CD100 proteins and active fragments thereof, i.e, peptides having a CD100 activity, such as human CD100. This invention also provides a preparation of CD100 or fragment thereof. An "isolated" protein is substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. In a preferred embodiment, the CD100 protein has an amino acid sequence shown in Figure 2, SEQ ID NO: 2. In other embodiments, the CD100 protein is substantially homologous or similar to Figure 2, SEQ ID NO: 2 and retains the functional activity of the protein of Figure 2, SEQ ID NO: 2 yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the CD100 protein is a protein which comprises an amino acid sequence at least 80 % overall amino acid sequence identity with the amino acid sequence of Figure 2, SEQ ID NO: 2. Preferably, the protein is at least 90 % identical to a protein having an amino acid sequence shown in Figure 2, SEQ ID NO: 2 or in Figure 14, SEQ ID NO: 9, more preferably at least 95 % identical to a protein having an amino acid sequence shown in Figure 2, SEQ ID NO: 2 or in Figure 14, SEQ ID NO: 9, even more preferably at least 98-99 % identical to a protein having an amino acid sequence shown in Figure 2, SEQ ID NO: 2 or in Figure 14, SEQ ID NO: 9.

An isolated CD100 protein may comprise the entire amino acid sequence of Figure 2, SEQ ID NO: 2 or a biologically active portion thereof. For example, an active portion of CD100 can comprise a mature form of CD100 in which a hydrophobic, amino-terminal signal sequence is absent. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for a CD100 activity as described in detail above. For example, a peptide having a CD100 activity may differ in amino acid sequence from the human CD100 depicted in Figure 2 (SEQ ID NO: 2), but such differences result in a peptide which functions in the same or similar manner as CD100. Thus, peptides having the ability to stimulate a leukocyte response, such as a B cell response, e.g., B cell aggregation, and which preferably has a semaphorin domain or an active portion thereof are within the scope of the invention. Preferred peptides of the invention include those which are further capable of inducing B cell differentiation, stimulating B cell survival, downregulating CD40 induced CD23 expression and/or costimulating T cell proliferation.

One embodiment provides a CD100 derived peptide having B cell binding activity, but lacking the ability to stimulate a leukocyte response, such as a B cell response. Such a peptide can be used to inhibit or block B cell differentiation or T cell proliferation in a

subject. Alternatively, a peptide having both B cell binding activity and the ability to deliver a stimulatory signal to B cells can be used to stimulate or enhance a B cell response, such as B cell aggregation and/or antibody production in a subject. Similarly, a peptide having T cell binding activity and the ability to deliver a stimulatory signal to T cells can be used to stimulate or enhance a T cell response, such as T cell proliferation. Various modifications of the CD100 protein to produce these and other functionally equivalent peptides are described in detail herein. The term "peptide" as used herein, refers to peptides, proteins and polypeptides.

A peptide can be produced by modification of the amino acid sequence of the human CD100 protein shown in Figure 2 (SEQ ID NO: 2) or murine CD100 protein shown in Figure 14 (SEQ ID NO: 9), such as a substitution, addition or deletion of an amino acid residue which is not directly involved in the function of CD100.

Peptides of the invention are typically at least about 30 amino acid residues in length, preferably at least about 40 amino acid residues in length, more preferably at least about 50 amino acid residues in length,and most preferably about 60 amino acid residues in length. Peptides having CD100 activity and including at least about 80 amino acid residues in length, at least about 100 amino acid residues in length, at least about 200, at least about 300, at least about 400, or at least about 500 or more amino acid residues in length are also within the scope of the invention. A preferred peptide includes an extracellular domain or portion thereof of the human CD100 antigen (e.g., about amino acid residues 22-734 or 24-734 of the sequence shown in Figure 2 (SEQ ID NO: 2). Other preferred peptides have an amino acid sequence represented by a formula:

$$X_n\text{-}Y\text{-}Z_m$$

where Y is selected from the group consting of: an extracellular domain comprising the signal peptide, which is preferably about amino acid residues 1-630 or 1-734 of the sequence shown in Figure 2 (SEQ ID NO: 2); a semaphorin domain, which is preferably about amino acid residues 42-553 of the sequence shown in Figure 2 (SEQ ID NO: 2); an Ig-like domain, which is preferably about amino acid residues 566-630 or 554-630 of the sequence shown in Figure 2 (SEQ ID NO: 2); a transmembrane domain, which is about amino acid residues 735-753 of the sequence shown in Figure 2 (SEQ ID NO: 2); and a cytoplasmic domain, which is about amino acid residues 754-862 of the sequence shown in Figure 2 (SEQ ID NO: 2). In the formula, X_n and Z_m are additional amino acid residues linked to Y by an amide bond. X_n and Z_m are amino acid residues selected from amino acids contiguous to Y in the amino acid sequence shown in Figure 2 (SEQ ID NO: 2). X_n is amino acid residues selected from amino acids contiguous to the amino terminus of Y in the sequence shown in Figure 2 (SEQ ID NO: 2). Z_m is amino acid residues selected from amino acids contiguous to th carboxy terminus of Y in the sequence shown in Figure 2

(SEQ ID NO: 2). According to the formula, n is a number from 0 to 753 (n=0-753) and m is a number from 0 to 234 (m=0-234). A particularly preferred peptide has an amino acid sequence represented by the formula X_n-Y-Z_m, where n=0 and m=0.

Other peptides within the scope of the invention include those encoded by the nucleic acids described herein.

Another embodiment of the invention provides a substantially pure preparation of a peptide having a CD100 activity. Such a preparation is substantially free of proteins and peptides with which the peptide naturally occurs in a cell or with which it naturally occurs when secreted by a cell.

The term "isolated" as used throughout this application refers to a nucleic acid, protein or peptide having an activity of a CD100 antigen substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. An isolated nucleic acid is also free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the organism from which the nucleic acid is derived.

The peptides and fusion proteins produced from the nucleic acid molecules of the present invention can also be used to produce antibodies specifically reactive with CD100 antigens. For example, by using a full-length CD100 antigen, such as an antigen having an amino acid sequence shown in Figure 2 (SEQ ID NO: 2), or a peptide fragment thereof, anti-protein/anti-peptide polyclonal antisera or monoclonal antibodies can be made using standard methods. A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the protein or peptide which elicits an antibody response in the mammal. The immunogen can be, for example, a recombinant CD100 protein, or fragment thereof, a synthetic peptide fragment or a cell that expresses a CD100 antigen on its surface. The cell can be for example, a splenic B cell or a cell transfected with a nucleic acid encoding a CD100 antigen of the invention (e.g., a /CD100 cDNA) such that the CD100 antigen is expressed on the cell surface. The immunogen can be modified to increase its immunogenicity. For example, techniques for conferring immunogenicity on a peptide include conjugation to carriers or other techniques well known in the art. For example, the peptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of antibodies.

Following immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera. To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art. For example, the hybridoma technique originally developed by Kohler and Milstein (*Nature* (1975) 256:495-497) as well as other t chniques such as the human B-cell hybridoma technique (Kozbar et al.,

Immunol. Today (1983) 4:72), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. *Monoclonal Antibodies in Cancer Therapy* (1985) Allen R. Bliss, Inc., pages 77-96), and screening of combinatorial antibody libraries (Huse et al., *Science* (1989) 246:1275). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the peptide and monoclonal antibodies isolated.

The term antibody as used herein is intended to include fragments thereof which are also specifically reactive with a peptide having the activity of a novel B lymphocyte antigen or fusion protein as described herein. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, $F(ab')_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments. The antibody of the present invention is further intended to include bispecific and chimeric molecules having an anti-CD100 antigen (i.e., CD100) portion.

When antibodies produced in non-human subjects are used therapeutically in humans, they are recognized to varying degrees as foreign and an immune response may be generated in the patient. One approach for minimizing or eliminating this problem, which is preferable to general immunosuppression, is to produce chimeric antibody derivatives, i.e., antibody molecules that combine a non-human animal variable region and a human constant region. Chimeric antibody molecules can include, for example, the antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. A variety of approaches for making chimeric antibodies have been described and can be used to make chimeric antibodies containing the immunoglobulin variable region which recognizes the gene product of the novel CD100 antigens of the invention. See, for example, Morrison et al., (1985), *Proc. Natl. Acad. Sci. U.S.A.* 81:6851 ; Takeda et al., (1985), *Nature* 314:452 , Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816.397; Tanaguchi et al., European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom Patent GB 2177096B. It is expected that such chimeric antibodies would be less immunogenic in a human subject than the corresponding non-chimeric antibody.

For human therapeutic purposes, the monoclonal or chimeric antibodies specifically reactive with a CD100 antigen as described herein can be further humanized by producing human variable region chimeras, in which parts of the variable regions, especially the conserved framework regions of the antigen-binding domain, are of human origin and only the hypervariable regions are of non-human origin. General reviews of "humanized" chimeric antibodies are provided by Morrison, S. L. (1985) *Science* 229:1202-1207 and by Oi et al. (1986) *BioTechniques* 4:214. Such altered immunoglobulin molecules may be made by any of several t chniques known in the art, (.g., Teng t al., (1983), *Proc. Natl.*

Acad. Sci. U.S.A., 80:7308-7312; Kozbor et al., (1983), *Immunology Today*, 4:7279; Olsson et al., (1982), *Meth. Enzymol.*, 92:3-16), and are preferably made according to the teachings of PCT Publication WO92/06193 or EP 0239400. Humanized antibodies can be commercially produced by, for example, Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain. Suitable "humanized" antibodies can be alternatively produced by CDR or CEA substitution (see U.S. Patent 5,225,539 to Winter; Jones et al. (1986) *Nature* 321:552-525; Verhoeyan et al. (1988) *Science* 239:1534; and Beidler et al. (1988) *J. Immunol.* 141:4053-4060). Humanized antibodies which have reduced immunogenicity are preferred for immunotherapy in human subjects. Immunotherapy with a humanized antibody will likely reduce the necessity for any concomitant immunosuppression and may result in increased long term effectiveness for the treatment of chronic disease situations or situations requiring repeated antibody treatments.

As an alternative to humanizing a monoclonal antibody from a mouse or other species, a human monoclonal antibody directed against a human protein can be generated. Transgenic mice carrying human antibody repertoires have been created which can be immunized with a CD100 antigen, such as human CD100. Splenocytes from these immunized transgenic mice can then be used to create hybridomas that secrete human monoclonal antibodies specifically reactive with a CD100 antigen (see, e.g., Wood et al. PCT publication WO 91/00906, Kucherlapati et al. PCT publication WO 91/10741; Lonberg et al. PCT publication WO 92/03918; Kay et al. PCT publication 92/03917; Lonberg, N. et al. (1994) *Nature* 368:856-859; Green, L.L. et al. (1994) *Nature Genet.* 7:13-21; Morrison, S.L. et al. (1994) *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Bruggeman et al. (1993) *Year Immunol* 7:33-40; Tuaillon et al. (1993) *PNAS* 90:3720-3724; and Bruggeman et al. (1991) *Eur J Immunol* 21:1323-1326).

Monoclonal antibody compositions of the invention can also be produced by other methods well known to those skilled in the art of recombinant DNA technology. An alternative method, referred to as the "combinatorial antibody display" method, has been developed to identify and isolate antibody fragments having a particular antigen specificity, and can be utilized to produce monoclonal antibodies that bind a CD100 antigen of the invention (for descriptions of combinatorial antibody display see e.g., Sastry et al. (1989) *PNAS* 86:5728; Huse et al. (1989) *Science* 246:1275; and Orlandi et al. (1989) *PNAS* 86:3833). After immunizing an animal with a CD100 antigen, the antibody repertoire of the resulting B-cell pool is cloned. Methods are generally known for directly obtaining the DNA sequence of the variable regions of a diverse population of immunoglobulin molecules by using a mixture of oligomer primers and PCR. For instance, mixed oligonucleotide primers corresponding to the 5' leader (signal peptide) sequences and/or framework 1 (FR1) sequences, as well as primer to a conserved 3' constant region primer can be used for PCR amplification of the heavy and light chain variable regions from a number of murine antibodies (Larrick et al. (1991) *Biotechniques* 11:152-156). A similar

strategy can also been used to amplify human heavy and light chain variable regions from human antibodies (Larrick et al. (1991) *Methods: Companion to Methods in Enzymology* 2:106-110).

In an illustrative embodiment, RNA is isolated from activated B cells of, for example, peripheral blood cells, bone marrow, or spleen preparations, using standard protocols (e.g., U.S. Patent No. 4,683,202; Orlandi, et al. *PNAS* (1989) 86:3833-3837; Sastry et al., *PNAS* (1989) 86:5728-5732; and Huse et al. (1989) *Science* 246:1275-1281.) First-strand cDNA is synthesized using primers specific for the constant region of the heavy chain(s) and each of the κ and λ light chains, as well as primers for the signal sequence. Using variable region PCR primers, the variable regions of both heavy and light chains are amplified, each alone or in combinantion, and ligated into appropriate vectors for further manipulation in generating the display packages. Oligonucleotide primers useful in amplification protocols may be unique or degenerate or incorporate inosine at degenerate positions. Restriction endonuclease recognition sequences may also be incorporated into the primers to allow for the cloning of the amplified fragment into a vector in a predetermined reading frame for expression.

The V-gene library cloned from the immunization-derived antibody repertoire can be expressed by a population of display packages, preferably derived from filamentous phage, to form an antibody display library. Ideally, the display package comprises a system that allows the sampling of very large diverse antibody display libraries, rapid sorting after each affinity separation round, and easy isolation of the antibody gene from purified display packages. In addition to commercially available kits for generating phage display libraries (e.g., the Pharmacia *Recombinant Phage Antibody System*, catalog no. 27-9400-01; and the Stratagene *SurfZAP*™ phage display kit, catalog no. 240612), examples of methods and reagents particularly amenable for use in generating a diverse antibody display library can be found in, for example, Ladner et al. U.S. Patent No. 5,223,409; Kang et al. International Publication No. WO 92/18619; Dower et al. International Publication No. WO 91/17271; Winter et al. International Publication WO 92/20791; Markland et al. International Publication No. WO 92/15679; Breitling et al. International Publication WO 93/01288; McCafferty et al. International Publication No. WO 92/01047; Garrard et al. International Publication No. WO 92/09690; Ladner et al. International Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum Antibod Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffths et al. (1993) *EMBO J* 12:725-734; Hawkins et al. (1992) *J Mol Biol* 226:889-896; Clackson et al. (1991) *Nature* 352:624-628; Gram et al. (1992) *PNAS* 89:3576-3580; Garrad et al. (1991) *Bio/Technology* 9:1373-1377; Hoogenboom et al. (1991) *Nuc Acid Res* 19:4133-4137; and Barbas et al. (1991) *PNAS* 88:7978-7982.

In certain embodiments, the V region domains of heavy and light chains can be expressed on the same polypeptide, joined by a flexible linker to form a single-chain Fv fragment, and the scFV gene subsequently cloned into the desired expression vector or phage genome. As generally described in McCafferty et al., *Nature* (1990) 348:552-554, complete V_H and V_L domains of an antibody, joined by a flexible $(Gly_4\text{-}Ser)_3$ linker can be used to produce a single chain antibody which can render the display package separable based on antigen affinity. Isolated scFV antibodies immunoreactive with a peptide having activity of a CD100 antigen can subsequently be formulated into a pharmaceutical preparation for use in the subject method.

Once displayed on the surface of a display package (e.g., filamentous phage), the antibody library is screened with a CD100 antigen, or peptide fragment thereof, to identify and isolate packages that express an antibody having specificity for the CD100 antigen. Nucleic acid encoding the selected antibody can be recovered from the display package (e.g., from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques.

The antibodies of the current invention can be used therapeutically to inhibit a B cell response through blocking receptor:ligand interactions necessary for transduction of a CD100 ligand-associated signal in the B cell. These so-called "blocking antibodies" can be identified by their ability to inhibit a B cell response when added to an *in vitro* assay as described herein. Antibodies of the invention can also be used to block a T cell response, such as T cell proliferation. Alternatively, the antibodies of the invention can be stimulatory antibodies, and stimulate a B cell and/or a T cell response.

The polyclonal or monoclonal antibodies of the current invention, such as an antibody specifically reactive with a recombinant or synthetic peptide having a CD100 activity can also be used to isolate the native CD100 antigen from cells. For example, antibodies reactive with the peptide can be used to isolate the naturally-occurring or native form of CD100 from activated B lymphocytes by immunoaffinity chromatography. In addition, the native form of cross-reactive CD100-like molecules can be isolated from B cells or other cells by immunoaffinity chromatography with an anti-CD100 antibody.

IV. Uses and Methods of the Invention

The invention further pertains to methods for modulating a leukocyte response in which a leukocyte is contacted *in vitro* or *in vivo* with an agent which modulates a CD100 ligand-associated signal. The term "leukocyte" is intended to include any cell of the blood which is not a red blood cell and includes lymphocytes, granulocytes, and monocytes. A preferred leukocyte is a lymphocyte, such as a B cell or a T cell.

The term "B cell" is intended to include a B lymphocyte that is at any state of maturation. Thus, the B cell can be a progenitor cell, a pre-B cell, an immature B cell, a mature B cell, a blast cell, a centroblast, a centrocyte, an activated B cell, a memory B cell,

or an antibody secreting plasma cell. A preferred B cell is an activated B cell, i.e, a B cell which has encountered an antigen. The term "B cell response" is intended to include a response of a B cell to a stimulus. The stimulus can be a soluble stimulus such as an antigen, a lymphokine, or a growth factor or a combination thereof. Alternatively, the stimulus can be membrane bound molecule, such as a receptor on T helper (Th) cells, e.g., CD28, CTLA4, gp39, or an adhesion molecule. Since a change in a B cell, such as a change occuring during the process of B cell maturation or activation is mediated by extracellular factors and membrane bound molecules, a response of a B cell is intended to include any change in a B cell, such as a change in stage of differentiation, secretion of factors, e.g., antibodies. Thus, a modulation of a B cell response can be a modulation of B cell aggregation, a modulation of B cell differentiation, such as differentiation into a plasma cell or into a memory B cell, or a modulation of cell viability. In a preferred embodiment, the invention provides a method for stimulating the differentiation of a B cell from a lymphobast to a centrocyte. In another preferred embodiment, the invention provides a method for modulating B cell aggregation, such as homotypic B cell aggregation. In another embodiment, the invention provides a method for modulating B cell survival. In yet another preferred embodiment, the invention provides a method for modulating production of antibodies by B cells. In a further embodiment, the invention provides a method for modulating proliferation of B cells.

In one embodiment, the invention provides a method for stimulating a T cell response. A T cell response is intended to include stimulation of any change in the T cell that results from interaction with a soluble or membrane bound factor. Thus, a T cell response can be T cell proliferation, T cytotoxic activity, secretion of cytokines, differentiation or any T cell effector function.

In a preferred embodiment, the invention provides a method for stimulating T cell proliferation in the presence of a primary activation signal, comprising contacting a T cell with a CD100 antigen or active fragment thereof and a primary activation signal. The T cell can be a CD4+ T cell, a CD8+ T cell, a T helper cell (Th), or a cytotoxic T cell (Tc). Interaction between the T cell receptor (TCR)/CD3 complex and antigen presented in conjunction with either major histocompatibility complex (MHC) class I or class II molecules on an antigen-presenting cell initiates a series of biochemical events termed antigen-specific T cell activation. The term "T cell activation" is used herein to define a state in which a T cell response has been initiated or activated by a primary signal, such as through the TCR/CD3 complex, but not necessarily due to interaction with a protein antigen. A T cell is activated if it has received a primary signaling event which initiates an immune response by the T cell.

T cell activation can be accomplished by stimulating the T cell TCR/CD3 complex or via stimulation of the CD2 surface protein. An anti-CD3 monoclonal antibody can be used to activate a population of T cells via the TCR/CD3 complex. Although a number of

anti-human CD3 monoclonal antibodies are commercially available, OKT3 prepared from hybridoma cells obtained from the American Type Culture Collection or monoclonal antibody G19-4 is preferred. Similarly, binding of an anti-CD2 antibody will activate T cells. Stimulatory forms of anti-CD2 antibodies are known and available. Stimulation through CD2 with anti-CD2 antibodies is typically accomplished using a combination of at least two different anti-CD2 antibodies. Stimulatory combinations of anti-CD2 antibodies which have been described include the following: the T11.3 antibody in combination with the T11.1 or T11.2 antibody (Meuer, S.C. et al. (1984) *Cell* 36:897-906) and the 9.6 antibody (which recognizes the same epitope as T11.1) in combination with the 9-1 antibody (Yang, S. Y. et al. (1986) *J. Immunol.* 137:1097-1100). Other antibodies which bind to the same epitopes as any of the above described antibodies can also be used. Additional antibodies, or combinations of antibodies, can be prepared and identified by standard techniques.

A primary activation signal can also be provided by a polyclonal activator. Polyclonal activators include agents that bind to glycoproteins expressed on the plasma membrane of T cells and include lectins, such as phytohemaglutinin (PHA), concanavalin (Con A) and pokeweed mitogen (PWM).

A primary activation signal can also be delivered to a T cell through use of a combination of a protein kinase C (PKC) activator such as a phorbol ester (e.g., phorbol myristate acetate) and a calcium ionophore (e.g., ionomycin which raises cytoplasmic calcium concentrations). The use of these agents bypasses the TCR/CD3 complex but delivers a stimulatory signal to T cells. These agents are also known to exert a synergistic effect on T cells to promote T cell activation and can be used in the absence of antigen to deliver a primary activation signal to T cells.

Accordingly, the invention provides a method for stimulating antigen specific T cell proliferation by contacting a T cell with a stimulatory form of a CD100 antigen and a primary activation signal provided by an antigen presented in the context of a major histocompatibility complex antigen, such as on an antigen presenting cell. The invention further provides a method for stimulating proliferation of T cells in a non antigen specific manner by contacting the T cell with a CD100 antigen and an agent which provides a non antigen specific primary activation signal, such as an anti-CD3 antibody, a polyclonal activator, a combination of a PKC activator and a calcium ionophore, or an agent which stimulates CD2.

The invention provides methods for modulating a leukocyte response. The term "modulating" is intended to include stimulation or inhibition of a leukocyte response. In one embodiment, the invention provides a method for stimulating B cell aggregation. In another embodiment, the invention provides a method for inhibiting B cell aggregation. Also within the scope of the invention are methods for stimulating stimulating T cell proliferation and methods for inhibiting T cell proliferation.

In one embodiment of the invention, a leukocyte response is modulated by contacting leukocytes with an agent which modulates a CD100 ligand-associated signal. The term "CD100 ligand-associated signal" is intended to include an intracellular signal which is induced by contact of the natural form of CD100 with its ligand on an immune cell, such as a leukocyte and which results in the biological effects induced by such a contact, e.g., stimulation of B cell aggregation, stimulation of differentiation, increased cell viability, reduction of CD23 expression, and/or stimulation of T cell proliferation. The CD100 ligand-associated signal can be mediated by one receptor, or by several different receptors. The term "CD100 receptor" is used herein interchangeably with the term "CD100 ligand" and is intended to include a surface molecule or a secreted molecule with which a CD100 antigen interacts. Such an interaction can be a "positive interaction", i.e., resulting in transduction of a CD100 ligand-associated signal and the resulting biological effects. An interaction of a CD100 molecule with one of its receptors or ligands can also be a "negative interaction", such that upon interaction of the receptor with a CD100 molecule, no CD100 ligand-associated signal is transduced. Thus, a CD100 molecule may interact with more than one receptor, and the outcome of the interaction with the different receptors may be different according to the specific receptor. Specific types of leukocytes and other cells may have specific receptors. The outcome of the interaction of CD100 with one of its receptors may depend on the presence of other signals delivered to the leukocyte. One possible receptor for CD100 is CD100 itself, or a molecule having a CD100 activity and/or being structurally related to CD100.

A. Agents which stimulate a CD100 ligand-associated signal

In one embodiment of the invention, the agent which modulates a CD100 ligand-associated signal is an agent which stimulates a CD100 ligand-associated signal. A CD100 ligand-associated signal can be stimulated in a leukocyte by contact of the leukocyte with a stimulatory form of CD100. Alternatively, a CD100 ligand-associated signal can be stimulated by an agent which is not a form of CD100, but which mimicks the effects of a stimulatory form of CD100 and interacts with the CD100 ligand on the leukocyte. In yet another embodiment, a CD100 ligand-associated signal is stimulated by contacting a leukocyte with an agent which does not interact with CD100, but which mimicks a CD100 ligand-associated signal.

In a preferred embodiment of the invention, the stimulatory agent is a stimulatory form of a CD100 molecule. The term "stimulatory form of a CD100 molecule" is intended to include an agent which is a CD100 antigen, or molecule derived therefrom and which interacts with a leukocyte, such that a CD100 ligand-associated signal is transduced in the leukocyte.

A preferred CD100 stimulatory form of CD100 is a membrane bound form of CD100. or a form of CD100 linked to a solid phase surface. It has been shown (Exampl s

4 and 5 herein) that contacting a population of B cells with NIH-3T3 cells expressing CD100 results in augmentation of cell clustering and B cell survival. Thus, the invention provides methods for stimulating B cell aggregation, B cell differentiation, and increased B cell survival comprising contacting a population of B cells with cells that express CD100 molecules on their surface. It has further been shown herein that contacting a population of T cells with NIH-3T3 expressing CD100 in the presence of anti-CD3 antibodies results in stimulation of T cell proliferation. Accordingly, the invention also provides methods for stimulating T cell proliferation, comprising contacting a population of T cells with cells that express CD100 and a primary activation signal. Such cells can be cells which naturally express CD100 on their suface. Alternatively, the CD100 cells for practicing the invention can be cells that have been transfected to express a stimulatory form of CD100. In yet another embodiment, CD100 positive cells are obtained by linking a CD100 molecule to the cell surface, e.g., using cross-linking reagents. A stimulatory form of CD100 can also be linked to a solid phase support, such as a bead. The stimulatory form of a CD100 molecule can be the full length protein. Alternatively, the stimulatory form of CD100 is a stimulatory fragment of CD100. A stimulatory fragment of a CD100 molecule is a fragment of a CD100 molecule which upon interaction with B cells results in stimulation of a B cell response, such as B cell aggregation. Nucleic acids that can be used for preparing stimulatory forms of CD100 as well as methods for expressing these nucleic acids are described above. An assay for confirming that a form of CD100 is a stimulatory form of CD100 is also described above and in the Example section. Briefly, in one such assay, cells containing CD100 on their surface are added to a culture of B cells, and the effect on B cell aggregation is monitored either macroscopically or microscopically. A stimulatory form of a CD100 antigen can also be identified by performing a proliferation assay of T cells incubated in the presence of the form of CD100 to be tested and an anti-CD3 antibody. Alternatively, T cell proliferation can be measured by determining the amount of cytokines, such as IL-2 and IL-4 secreted by the T cells, such as by an ELISA assay. Methods for measuring T cell proliferation are known in the art and can be found for example in the published PCT applications Number PCT/US94/06255 and PCT/US94/08423.

Another preferred stimulatory form of CD100 is a soluble form of CD100. A soluble stimulatory form of CD100 can be prepared by recombinant techniques well known in the art. For example, for preparing a soluble stimulatory form of a CD100 molecule shown in Figure 2, a nucleic acid fragment having a sequence shown in Figure 1 (SEQ ID NO: 1) comprising the nucleic acid encoding the semaphorin domain and the Ig-like domain, i.e., about amino acid residues 22-630, 24-630, 22-731, 24-731, 42-630, or 42 to 731 of SEQ ID NO: 1, but not comprising the transmembrane and cytoplasmic domains is expressed in a host cell and purified according to methods known in the art. In a preferred embodiment, the soluble stimulatory form of a CD100 protein comprises a second peptide

which modifies the first peptide, such as to increase its stability. A preferred second peptide is an immunoglobulin fragment. These are further described herein.

Other agents which stimulate a CD100 ligand-associated signal in a cell include agents which mimmick the action of a stimulatory form of CD100 on a cell. Such agents include small molecules which interact with a CD100 receptor or cell surface structure and deliver to the B cell a CD100 ligand-associated signal. Small molecules can be isolated from libraries of small molecules, such as combinatorial libraries, using *in vitro* assays, such as those described above for identifying fragments of CD100 which are capable of inducing a CD100-signal.

Methods within the scope of the invention pertain to the isolation of receptor(s) for CD100 molecules. Accordingly, additional agents which stimulate a CD100 ligand-associated signal in a B cell include agents which interact with the CD100 receptor and deliver a CD100 ligand-associated signal. Preferred stimulatory agents interacting with a CD100 receptor are antibodies to CD100 receptors which deliver to the B cell a CD100 ligand-associated signal. Such antibodies can be prepared as described herein for the preparation of anti-CD100 antibodies. Other agents which stimulate a CD100 ligand-associated signal include constitutively activated forms of CD100 receptors, that can be obtained, for example, by modifying the intracellular domain of the receptor, such that the modified receptor constitutively delivers a CD100 ligand-associated signal to the cell.

The interaction of a CD100 antigen with a leukocyte may result in stimulation of a leukocyte response through a signal delivered to the leukocyte from the CD100 receptor. Alternatively, the interaction of a leukocyte with a cell having a CD100 molecule on its surface may result in secretion of a soluble factor from the leukocyte or the cell having a CD100 molecule on its surface which then stimulates a leukocyte response. Thus, such a soluble factor could also be used as an agent which stimulates a CD100 ligand-associated signal and is also within the scope of the invention. Methods for isolating such a soluble factor are also within the scope of the invention.

In the instance where CD100 is its own receptor, stimulatory agents could be agents which interact with CD100, such as antibodies to CD100, such as the antibodies BD16 (Herold C. et al, (1994), *Int. Immunol.* 7, 1.), BB18 (Bougeret C., et al., (1992), *J. Immunol.* 148, 318.), and F937G2 (Seed B., (1987), *Nature* 329, 840.). Antibodies which stimulate CD100 can be prepared according to methods described herein.

B. Agents which inhibit a CD100 ligand-associated signal

In another embodiment of the invention, the agent which modulates a CD100 signal in a leukocyte is an agent which inhibits a CD100 ligand-associated signal. Inhibitory agents include agents which block stimulatory agents from delivering a CD100 ligand-associated signal and agents which deliver a negative, or inhibitory signal to the cell. Such agents are termed herein "CD100 inhibitors".

Agents which block the transduction of a CD100 ligand-associated signal include agents which interact with a CD100 receptor but fail to deliver a CD100 ligand-associated signal, thereby preventing delivery of a CD100 ligand-associated signal by stimulatory agents. Preferred inhibitory agents include inhibitory forms of CD100 molecules. Such inhibitory forms can, e.g., bind a CD100 receptor, fail to deliver a positive signal and thereby inhibit a CD100 ligand-associated signal. Alternatively, the inhibitory form of CD100 could deliver a negative signal, further inhibiting a B cell response. Inhibitory forms of CD100 can be modified forms of CD100 molecules, fragments of CD100 molecules, or modified fragments. Inhibitory forms of CD100 antigens can be prepared by methods similar to those described herein for the preparation of stimulatory forms of CD100. An inhibitory form of CD100 will preferably comprise the extracellular domain of a CD100 molecule, or at least a portion of the extracellular domain that is necessary for interacting with the CD100 receptor. CD100 molecules or fragments thereof that retain binding capacity to CD100 receptors can then be mutated, such as by site directed mutagenesis for obtaining a form of CD100 or fragment thereof that retains binding capacity but fails to deliver a CD100 ligand-associated signal. The inhibitory efficiency of the inhibitory forms of CD100 can be determined, e.g., in an *in vitro* assay wherein B cells are incubated in the presence of a stimulatory form of CD100, such as NIH-3T3 cells expressing a full length CD100 molecule on the cell surface. Various amounts of the inhibitory form of CD100 to be tested are added to this assay and a B cell response, such as B cell aggregation is measured. An inhibitory form of CD100 will inhibit or at least reduce B cell aggregation. An inhibitory form of CD100 can also be identified as a form of CD100 which, when added to a culture of T cells in the presence of a primary T cell activation signal and a stimulatory form of CD100 inhibits T cell proliferation.

Additional agents within the scope of the invention which block the transduction of a CD100 ligand-associated signal include agents which interact with CD100 and thereby prevents its interaction with a CD100 receptor. Preferred inhibitory forms of CD100 include antibodies to CD100, such as blocking antibodies. Thus, blocking antibodies to CD100 will interact with CD100 and block its interaction with its ligand, thus inhibiting transduction of a signal from the CD100 ligand. Blocking antibodies can be prepared according to methods known in the art, using, for example, a soluble form of an extracellular domain of CD100 as the antigen. Also within the scope of the invention are soluble forms of CD100 receptors or fragments thereof or modified forms thereof which are capable of interacting with CD100 and blocking its interaction with a CD100 receptor on a cell. The soluble forms of CD100 receptors may also be linked to a solid phase surface. These soluble or membrane bound forms of CD100 receptors can be prepared and modified similarly to the soluble CD100 molecules described herein.

Additional inhibitory agents include small molecules. Thus, it is possible to isolate small molecules which inhibit a signal through CD100 from a library of small molecules using, for example, the assay described above. These molecules can block a CD100 ligand-associated signal by either interacting with CD100 or with its receptor, such that the interaction between CD100 and its receptor is inhibited. Alternatively, the small molecule may interact directly with the CD100 receptor and deliver a negative signal.

Preferred methods for inhibiting a CD100 ligand-associated signal in a leukocyte include contacting a leukocyte with a combination of at least two inhibitory agents. In a particularly preferred embodiment, the method includes contacting a leukocyte with at least one inhibitory agent which interacts with CD100 and with at least one inhibitory agent which interacts with a CD100 receptor.

Other inhibitory agents include CD100 antisense molecules and ribozymes. Thus, delivery of a CD100 ligand-associated signal by a cell expressing CD100 can be inhibited in a cell, such as a B cell or a T cell, by introducing and/or expressing CD100 antisense molecules or ribozymes. Antisense molecules and ribozymes are further described herein. Alternatively, a CD100 ligand-associated signal can be blocked in a cell, such as a B cell or a T cell by blocking the expression of a ligand for CD100 in the cell. Blocking or at least reducing the expression of a CD100 ligand can be obtained by introducing and/or expressing in the T or B cell antisense molecules interacting with a CD100 ligand.

C. Modulation of a B cell response by modulation of a CD100 ligand-associated signal and a CD40 signal

The invention further provides methods for moduling a B cell response comprising contacting the population of cells with a first agent which modulates a CD100 ligand-associated signal and a second agent which modulates a CD40 signal. Similarly to CD100 stimulation, resting B cells receiving a CD40 mediated contact dependent signal from CD40L on activated T cells are stimulated to aggregate, cluster, and proliferate (Banchereau J. et al., (1994), *Annu. Rev. Immunol.* 12, 881; Clark E. A. et al, (1994), *Nature* 367, 425. As also shown herein (Example 4), CD40 ligand (CD40L) transfectants (t-CD40L) induce B cells to aggregate and form clusters. It has been shown herein that B cell aggregation and cell survival induced by stimulation of B cells through CD100 is synergistically increased by simultaneously stimulating the cells through CD40. Thus, in a preferred method of the invention, a population of cells is contacted with an agent which provides a stimulatory signal through CD100 and an agent which provides a stimulatory signal through CD40. Also within the scope of the invention are methods for inhibiting a B cell response comprising contacting B cells with at least one first agent which inhibits a CD100 signal and at least one second agent which inhibits a CD40 signal, such that a B cell response is inhibited.

D. Additional methods of use of CD100 antigens

In another embodiment of the invention, the B cells which are contacted with an agent which stimulates a CD100 ligand-associated signal are further contacted with T cells. Thus, since CD100 provides a costimulatory signal to T cells, these T cells will provide help to the B cells for further stimulating a B cell response. In yet another embodiment, the population of cells comprising B and T cells further comprises at least one primary activation signal, such as an antigen, for example on an antigen presenting cell, or an anti-CD3 antibody.

The methods of the invention can be practiced *in vitro* or *in vivo*. For example a stimulatory form of a CD100-like molecule can be added to a culture of B cells, such as a hybridoma culture, such that the production of antibodies by the hybridomas is increased. Alternatively, the molecules of the invention can be added to a population of T cells, such that their proliferation *in vitro* is stimulated. The molecules of the invention can also be added to a culture of primary B cells or T cells obtained from an individual for stimulating their differentiation and proliferation, respectively.

V. Applications of the Invention

The invention provides a method for modulating a leukocyte response comprising contacting the leukocytes with an agent which modulates a CD100 ligand-associated signal in the leukocytes. In one embodiment, the invention provides a method for stimulating a B cell response. Stimulation of a B cell response can result in increased B cell aggregation, increased B cell differentiation and/or increased B cell survival. The B cells can, for example, be stimulated to differentiate from a lymphoblast to a centroblast or centrocyte and thereby stimulate the differentiation of B cells into either antibody secreting plasma cells or memory B cells. In another embodiment, the invention provides a method for stimulating a T cell response, such as T cell proliferation. In a preferred embodiment, the invention provides a method for stimulating a B cell response and a T cell response, such as T cell proliferation. Thus, the presence of a stimulatory form of CD100 in a population of cells comprising both B cells and T cells should further amplify the B cell response stimulated by CD100. It will be appreciated that it is particularly advantageous to stimulate both B cells and T cells for most applications.

In one embodiment, the invention provides a method of vaccination. Accordingly, a subject in need of vaccination is given a dose of at least one antigen in the presence of a therapeutically effective amount of an agent which stimulates a CD100 ligand-associated signal. The antigen and the stimulatory agent are preferably administered simultaneously. Alternatively, the antigen and the stimulatory agent are administered separately, such that, for example, the antigen is administered prior to the stimulatory agent. In a preferred embodiment, the subject is subsequently boosted at least once with additional doses of antigen and the stimulatory agent. The antigen can be any molecule or structure against

which it is desirable to have a strong and rapid immune reaction. Thus, antigens within the scope on the invention include viral antigens, bacterial antigens, tumor antigens, or any antigens, or combination of antigens from any organism. An agent which stimulates a CD100 ligand-associated signal can be added to any classic vaccine composition for preventing an infection in a subject.

In a preferred embodiment, the vaccine formulation further comprises a therapeutically effective dose of an agent which stimulates CD40. Thus, the subject is contacted with at least one antigen, an agent which stimulates a CD100 ligand-associated signal and a an agent which stimulates CD40. In a preferred embodiment, the CD40 and the agent which stimulates a CD100 ligand-associated signal are administered together to the subject, which is also preferably administered together with the antigen(s). Further boosting of the subject with the antigen(s) is preferably peformed by administering the antigen(s) and the stimulatory CD40 agent and/or the agent which stimulates a CD100 ligand-associated signal.

An agent which stimulates a CD100 ligand-associated signal can also be used for treating disorders in which boosting of a B cell response is beneficial. Such disorders include infections by pathogenic microorganisms, such as bacteria, viruses, and protozoans. Preferred disorders for treating according to the method of the invention include extracellular bacterial infections, wherein bacteria are eliminated through opsonization and phagocytosis or through activation of the complement. Other preferred infections that can be treated according to the method of the invention include viral infections, including infections with an Epstein-Barr virus or retroviruses, e.g., a human immunodeficiency virus.

In another embodiment of the invention, an agent which stimulates a CD100 ligand-associated signal can be administered to a subject having an antibody deficiency disorder resulting, for example, in recurrent infections and hypogammaglobulinemia (Ochs et al. (1989) Disorders in Infants and Children, Stiehm (ed.) Philadelphia, W.B. Sanders, pp 226-256). These disorders include common variable immunodeficiency (CVI), hyper-IgM syndrome (HIM), and X-linked agammaglobulinemia (XLA). Some of these disorders, e.g., HIS, are caused by a mutation in the CD40 ligand, gp39, on the T cell and administration of an agent which stimulates a CD100 ligand-associated signal would thus compensate for at least some of the B cell deficiencies, such as stimulation of B cell differentiation.

Furthermore, upregulation of a B cell response is also useful for treating a subject with a tumor. In one embodiment, an agent which stimulates a CD100 ligand-associated signal is administered at the site of the tumor. In another embodiment, an agent which stimulates a CD100 ligand-associated signal is administered systemically.

In another embodiment, the invention provides a method for stimulating B cells in culture, such as hybridoma cells. In a preferred embodiment, stimulation of the population

of B cells results in increased antibody production. Thus, an agent which stimulates a CD100 ligand-associated signal can be added at an effective dose to a B cell culture, such as a hybridoma, such that antibody production by the B cells is enhanced. The effective dose of the agent which stimulates a CD100 ligand-associated signal to be added to the culture can easily be determined experimentally. This can be done, for example, by adding various amounts of the agent to a constant amount of B cells, and by monitoring the amount of antibody produced, e.g., by ELISA. The effective dose corresponds to the dose at which highest amounts of antibodies are produced.

In yet another embodiment, an agent which stimulates a CD100 ligand-associated signal is administered together with a hybridoma into the peritoneal cavity of a mouse, such that the amount of antibody produced by the hybridoma is increased.

In another embodiment of the invention, a T cell is contacted with an agent which stimulates a CD100 ligand-associated signal and a primary activation signal, such that T cell proliferation is increased. The primary activation signal can be an antigen, or a combination of antigens, such that proliferation of one or more clonal populations of T cells is stimulated. Alternatively the primary activation signal can be a polyclonal agent, such as an antibody to CD3, such that T cell proliferation is stimulated in a non clonal manner.

In one embodiment, the invention provides a method for expanding a population of T cells *ex vivo*. Accordingly, primary T cells obtained from a subject are incubated with an agent which stimulates CD100 ligand-associated signal and a primary activation signal. Following activation and stimulation of the T cells, the progress of proliferation of the T cells in response to continuing exposure to the agent which stimulates a CD100-induced signal is monitored. When the rate of T cell proliferation decreases, the T cells are reactivated and restimulated, such as with additional anti-CD3 antibody and an agent which stimulates a CD100-induced signal in the T cell, to induce further proliferation. The monitoring and restimulation of the T cells can be repeated for sustained proliferation to produce a population of T cells increased in number from about 100- to about 100,000-fold over the original T cell population. Methods for stimulating the expansion of a population of T cells are further described in the published PCT application PCT/US94/06255 and can be practiced by using an agent which is capable of stimulating a CD100 ligand-associated signal as the costimulatory molecule. In a preferred embodiment, T cell expansion is obtained by incubating the T cells with a primary activation signal and at least two costimulatory signals, such as a molecule providing a costimulatory signal through a CD28 or CTLA4 receptor, such as B7-1 or B7-2, and a costimulatory molecule that stimulates a CD100 ligand-associated signal.

The method of the invention can be used to expand selected T cell populations for use in treating an infectious disease or cancer. The resulting T cell population can be genetically transduced and used for immunotherapy or can be used for *in vitro* analysis of infectious agents such as HIV. Proliferation of a population of $CD4^+$ cells obtained from

an individual infected with HIV can be achieved and the cells rendered resistant to HIV infection. Following expansion of the T cell population to sufficient numbers, the expanded T cells are restored to the individual. The expanded population of T cells can further be genetically transduced before restoration to a subject. Similarly, a population of tumor-infiltrating lymphocytes can be obtained from an individual afflicted with cancer and the T cells stimulated to proliferate to sufficient numbers and restored to the individual. In addition, supernatants from cultures of T cells expanded in accordance with the method of the invention are a rich source of cytokines and can be used to sustain T cells *in vivo* or *ex vivo*.

In another embodiment of the invention, T cell proliferation is stimulated *in vivo*. In a preferred embodiment, an agent which stimulates a CD100 ligand-associated signal in the T cell is administered to a subject, such that T cell proliferation in the subject is stimulated. The subject can be a subject that is immunodepressed, a subject having a tumor, or a subject infected with a pathogen. The agent of the invention can be administered locally or systemically. The agent can be administered in a soluble form or a membrane bound form. Additional applications for an agent capable of providing a costimulatory signal to T cells, such that their proliferation is stimulated, are described in the published PCT applications PCT/US94/13782 and PCT/US94/08423, the content of which are incorporated herein by reference.

The invention further provides inhibitors of a CD100 ligand-associated signal. In a specific embodiment, an inhibitory CD100 agent is used for prolonging graft survival. Thus, a therapeutically effective dose of an inhibitor of a CD100-signal is administered to a recipient of an allotypic or xenotypic graft, such that graft survival is prolonged at least in part by reducing a B cell response to the graft. In a preferred embodiment, the inhibitor of a CD100 ligand-associated signal is administered prior to transplantation of the graft, such that B cell responses are downregulated at the time of translplantation. Alternatively, the inhibitor of a CD100-signal can be administered at about the same time as transplantation of the graft or after transplantation of the graft. In preferred embodiments, the inhibitor of a CD100-signal is administered together with an inhibitor of a CD40-induced signal to further block B cell responses. Additional agents, such as agents which block T cell activation and clonal expansion, can also be administered to the graft recipient. Such agents include antibodies to B7-1, B7-2, a combination thereof, and CTLA4Ig.

In yet another embodiment of the invention, an inhibitor of a CD100 signal can be used to prevent graft versus host disease (GVHD). In a preferred embodiment, the donor bone marrow is incubated with an inhibitor of a CD100-signal prior to administration to the recipient. The treatment can further include administration of a therapeutically effective dose of a CD100 inhibitor to the bone marrow recipient prior to and/or after bone marrow transplantation. As described in the treatment for prolonging graft survival, the CD100 inhibitor can be used together with a CD40 inhibitor and/or with yet additional agents.

Inhibitors of CD100 can also be used to reduce B cell and/or T cell responses in autoimmune diseases which involve autoreactive B and/or T cells. Accordingly, administration of an inhibitor of CD100 to a subject can be used for treating a variety of autoimmune diseases and disorders having an autoimmune component, including diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, myasthenia gravis, systemic lupus erythematosis, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, including keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Crohn's disease, Graves ophthalmopathy, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis.

The efficacy of a CD100 inhibitor in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/*lpr/lpr* mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., *Fundamental Immunology*, Raven Press, New York, 1989, pp. 840-856).

It is possible to treat a subject having an allergy with an agent, such as an agent which blocks a CD100 ligand-associated signal, which inhibits or reduces production of IgE antibodies to thereby ameliorate the allergic reaction. Allergic reactions may be systemic or local in nature, depending on the route of entry of the allergen and the pattern of deposition of IgE on mast cells or basophils. Thus, in various embodiments for treating allergies, the agent is administered either systemically or locally. Alternatively, the allergen can be administeredtogether with an agent which blocks a CD40 signal. The treatment can further comprise adminstration of an agent which neutralizes IL-4, such as an anti-IL4 antibody.

Additional disorders for which the agents of the invention provide a treatment include lymphomas which are characterized by abnormal B cells. It has been shown by immunohistopathology that some lymphomas are characterized by high expression of CD100, whereas other types of lymphomas are characterized by low level expression of CD100. Lymphomas characterized by a block in B cell differentiation express low levels

of CD100. Thus, agents which stimulate a CD100 ligand-associated signal can be used to treat lymphomas which are characterized by a block in B cell differentiation, such that differentiation of the lymhoma cells is stimulated. Such lymphomas are characterized by small, well-differentiated lymphocytes and include low-grade lymphomas, such as small lymphocytic and follicular lymphomas. In one embodiment of the invention, an agent which stimulates a CD100 ligand-associated signal, such as a soluble stimulatory form of CD100, is administered to a subject having a low grade lymphoma, such that differentiation of the lymphoma cells is stimulated. Lymphomas characterized by an excessive proliferation and/or differentiation of B cell differentiation express high levels of CD100. Thus, inhibitors of CD100 can be used for treating a subject having a lymphoma characterized by an excessive proliferation and/or differentiation of B cells, such that their proliferation and/or differentiation is inhibited. Such lymphomas are characterized by larger, poorly differentiated lymphocytes and large lymphocytes with characteristics of a histiocyte or lymphoblast and include : intermediate grade lymphomas, such as follicular large cell, diffuse small cleaved cell, diffused mixed cell, and diffuse large cell lymphomas; high grade lymphomas, such as immunoblastic, small noncleaved (Burkitt's and non-Burkitt's), lymphoblastic, and true histiocytic lymphomas. In one embodiment, an inhibitor of CD100 is administered to a subject having a large cell lymphoma, such that proliferation and/or differentiation of the lymphoma cells is inhibited.

Additional applications for an agent capable of inhibiting a costimulatory signal in T cells, such that T cell proliferation is inhibited, are described in the published PCT applications PCT/US94/13782 and PCT/US94/08423, the content of which are incorporated herein by reference.

In preferred embodiments of the invention agents which modulate a CD100 ligand-associated signal are preferably administered to subject together with agents which modulate other costimulatory signals. For example, in a method for preventing graft rejection, it is preferable to administer to a subject having been transplanted an agent which inhibits a CD100 ligand-associated signal together with agents which inhibit a costimulatory signal delivered by a member of the B7 family of proteins, such as B7-1 and B7-2. In a preferred embodiment, an agent which blocks a CD100 associated signal is administered together with anti-B7-1 and/or anti-B7-2 antibodies, or CTLA4Ig. The method can further comprise administering to the subject additional agents, such as agents which inhibits the interaction of growth or differentiation factors with their receptors.

Also within the scope of the invention are diagnostic methods. It has been shown, for example, that B cell lymphomas are associated with a high level of CD100 expression. Thus, determining the level of CD100 expression in a lymph node section from a patient having enlarged lymph nodes using an agent that binds CD100 could be indicative of lymphoma. Detection of CD100 can also be performed using a nucleic acid probe that selectively recognizes the g ne encoding CD100. A specific probe can be a portion of a

nucleic acid having a SEQ ID NO: 1. Other disorders associated with a high level of CD100, a CD100 ligand, a CD100-like molecule or receptor thereof could similarly be detected using agents of the invention.

Since the semaphorin domain of CD100 shares some homology with other semaphorin family members, such as H-Sema III and M-Sema C, it is likely that CD100 interacts with at least some of these neurological semaphorins. However, since the semaphorins identified in the nervous system act as chemorepellants, it is possible that CD100 is repulsive towards these neurological semaphorins. Thus, whether CD100 or CD100 ligand(s) act as chemorepellants or chemoattractants vis a vis the neurological semaphorins, blocking the interaction between an immune semaphorin, or receptor thereof, and a neurologic semaphorin, or receptor therof, will result in modulation of the interaction between the immune and the nervous system. Such a modulation will be of interest in treating diseases characterized by an abnormal interaction between the immune and nervous systems.

Furthermore, if CD100 or its receptor(s) is capable of interacting with or repulsing neurologic semaphorins, the agents of the invention can be used for treating neurological diseases. Thus, depending on the disease to be treated, an agent of the invention which blocks or which stimulates interaction between neurons can be administered to a subject having the disease. Applications for CD100 agents or ligands in neurology include treatments for nerve damages, such as spinal cord injuries. The agents of the invention can also be used to direct regenerating neurons to their target.

Semaphorins are expressed during embryonic development. Thus, since CD100 is structurally related to at least some semaphorins, CD100 may also be involved in embryogenesis. Thus, CD100 agents could be useful in the regeneration of tissues or organs.

The invention can also be used to isolate one or more ligands of CD100 or CD100-like molecules. Various methods can be used for isolating ligands of CD100. A preferred method for isolating a CD100 ligand comprises screening of an expression library with CD100, such as a soluble form of CD100, according to methods known in the art. The expression library can be prepared from various tissues, preferably leukocytes, and even more preferably B lymphocytes. Thus, according to this method, a cDNA expression library is constructed using mRNA from B lymphocytes and the library is transfected into cells, preferably COS cells. The transfected cells are then incubated with a soluble form of CD100 that is tagged, e.g., with a biotin molecule. Cells binding CD100 are then detected by incubating the cells with a secondary reagent that is labelled and the cells are sorted by FACS analysis or using magnetic beads coated with a reagent that reacts with the CD100 labelled cells. The plasmids containing the cDNA are extracted, retransfected into COS cells, and rescreened with CD100. Following several rounds of screening, the plasmids are isolated and the insert is sequenced. A basic expression cloning techniqu has been

described by Seed and Aruffo, *Proc. Natl. Acad. Sci. USA*, 84:3365-3369 (1987) and Aruffo and Seed, *Proc. Natl. Acad. Sci. USA*, 84:8573-8577 (1987), although modifications to this technique may be necessary. The technique is also described in the published PCT application PCT/US94/08423.

VI. Pharmaceutical Compositions

The CD100 proteins and other agents described herein can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the protein or agent and a pharmaceutically acceptable carrier. As used herein the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

In one embodiment, the agents of the invention can be administered to a subject to modulate a B cell response in the subject, e.g., for stimulating the clearance of a pathogen from the subject. The agents are administered to the subjects in a biologically compatible form suitable for pharmaceutical administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the agents, e.g., protein to be administered in which any toxic effects are outweighed by the therapeutic effects of the agent. The term "subject" is intended to include living organisms in which an immune response can be elicited, e.g., mammals. Examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof. Administration of a therapeutically active amount of an agent of the present invention is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically active amount of a stimulatory form of a CD100 molecule, alone or together with a stimulatory form of a CD40 ligand, may vary according to factors such as the disease state, age, sex, and weight of the subject, and the ability of agent to elicit a desired response in the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The agent may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the agent may be coated in a material to protect it from the action of enzymes, acids and other natural conditions which may inactivate the agent. For example, solutions or suspensions used for parenteral,

intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

To administer an agent by other than parenteral administration, it may be necessary to coat the agent with, or co-administer the agent with, a material to prevent its inactivation. For example, a stimulatory form of a CD100-like molecule may be administered to a subject in an appropriate carrier or diluent co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP) and trasylol. Liposomes include water-in-oil-in-water emulsions as well as conventional liposomes (Strejan et al., (1984) *J. Neuroimmunol* 7:27). Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the agent in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the agent into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient (e.g., peptide) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and

directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

This invention is further illustrated by the following Exemplification which should not be construed as limiting. The contents of all references and published patent applications cited throughout this application are hereby incorporated by reference.

EXEMPLIFICATION

Example 1: Isolation of a cDNA encoding CD100

A cDNA encoding a human CD100 antigen was isolated by COS cell expression cloning using an anti-CD100 antibody. A cDNA library was constructed in pCDM8 (Aruffo A et al, (1987), *Proc. Natl. Acad. Sci. USA* 84, 8573; Seed B et al, (1987), *Proc. Natl. Acad. Sci. USA* 84, 3365) using poly (A)+RNA from PHA activated human T cells and size selected for inserts greater than 3 kb. The library was introduced into COS cells by DEAE-dextran transfection and CD100 expressing cells selected by two rounds of immunoselection and panning with CD100 mAb BD16 (Herold C. et al, (1994), *Int. Immunol.* 7, 1). Twelve of 16 plasmids isolated contained a 4.2kB Xba insert and COS cells individually transfected bound to BD16 but not to control Mouse IgG_1.

To confirm that the isolated 4.2 kb cDNA encodes CD100, COS cells were transiently transfected with the 4.2 kb CD100 cDNA, pCDM8 vector alone, and B7-1 cDNA. After 48 hours, cells were stained with antibodies against CD100 - BD16 (Herold C. et al, (1994), *Int. Immunol.* 7, 1), BB18 (Bougeret C et al., (1992), *J. Immunol.* 148, 318) and F937G2 (Seed B., (1987), *Nature* 329, 840), and an isotype matched B7-1 antibody, B1.1. Binding of the mAbs was detected by indirect immunofluorescence using goat anti-mouse IgG_1-phycoerythrin and analyzed by flow cytometry. Figure 5, which represents the results of the flow cytometry analysis, show that the COS cells transfected with the 4.2 kb cDNA bind specifically to three monoclonal antibodies (mAbs) directed against CD100 (Herold C et al, in *Leucocyte Typing V*, S. F. Schlossman, et al., Eds. (Oxford University Press, Oxford, 1995), vol. 1, pp. 50), but not to control mAbs. Non transfected COS cells do not stain with the anti-CD100 antibodies. These results indicate that the 4.2 kb cDNA encodes CD100.

Further confirmation that the isolated 4.2kb cDNA encodes CD100 was provided by immunoprecipitation experiments. CHO cells were stably transfected with CD100 cDNA or vector alone and ^{125}I labeled. Cell lysates were immunoprecipitated with BB18 antibody or a control antibody, IgG1, and separated on an 8.5% SDS-PAGE gel. The results indicate that a 150 kDa protein is immunoprecipitated from CHO cells transfected with CD100 and from activated T c lls, but not from untransfected CHO cells. Activated B

cells also express a 150 kDa protein that is immunoprecipitated with an anti-CD100 antibody.

The nucleotide sequence of CD100 was determined for both strands of the original 4.2 kb clone and another clone 4.2H which was isolated from the same library by hybridization. The sequencing was carried out using synthetic oligonucleotide primers and dye-labeled terminator/Taq polymerase chemistry, and analyzed on an automated fluorescent DNA sequencer (Applied Biosystems). The sequence of the CD100 cDNA reveals a single long open reading frame of 2.6 kb, which is represented in Figure 1. Initiation of translation is likely to occur at the second ATG which, unlike the first ATG, is in a favorable context for translation initiation (Kozak M., (1995), *Proc. Natl. Acad. Sci. USA* 92, 2662; Kozak M., (1989), *J. Cell. Biol.* 108, 229). The CD100 sequence is deposited in Genbank under accession number

A BLAST (Altschul S. F.et al, (1990), *J. Mol. Biol.* 215, 403) search of the protein database indicated that CD100 is a novel protein, which has some homology to the semaphorin gene family. The amino acid sequence deduced from the 4.2 kb CD100 cDNA was compared to sequences using CLUSTAL. Figure 2 represents a comparison of the H-Sema III and mouse Sema C (M-Sema C) amino acid sequences. CD100 shares 39% identity with H-sema III in the sema domain and 33% identity in the Ig-like domain, whereas the rest of the protein is strikingly divergent. As seen in Figure 3, the CD100 protein consists of a signal sequence followed by a sema domain, an Ig domain, a lysine-rich stretch of 104 amino acids, a hydrophobic transmembrane region and a cytoplasmic tail of 110 amino acids. A consensus site for tyrosine phosphorylation, KPALTGY at amino acid 813 in the cytoplasmic tail supports the predicted association of CD100 with a tyrosine kinase (Sidorenko S et al, "Identification of antigens associated with protein kinases by activation antigens panel mAb." S. F. Schlossman, et al., Eds., *Leucocyte Typing V* (Oxford University Press, Oxford, 1995), vol. 1; Rudd C et al., "Identification of antigens associated with protein kinases by activation antigen panel mAb." S. F. Schlossman, et al., Eds., *Leucocyte Typing V* (Oxford University Press, Oxford, 1995), vol. 1). CD100 contains 15 of the 16 conserved sema domain cysteines and 9 putative N-linked glycoslyation sites.

Thus, CD100 is a novel leukocyte semaphorin-like protein.

Example 2: CD100 is expressed on activated B and T cells and in non hematopoietic tissues

The level of CD100 mRNA was measured in several tissues by Northern blot analysis. 5 µg poly(A)+ RNA from activated B cells, Raji cells and activated T cells was separated by electrophoresis, transfered onto blots and hybridized with labeled 4.2 kb CD100 cDNA. These results indicate that CD100 expression is high in activated B and T cells and in Raji cells. Expression of CD100 is low in resting B and T cells. Furthermore,

this experiment showed the presence of two RNA species: one predominant species of about 4.5kb and a second species of about 9 kb.

Expression of CD100 was also analysed in various non hematopoietic tissues by Northern blot analysis. For this example, 2 μg of poly(A)+ RNA was separated, blotted and hybridized with labeled 4.2kb CD100 cDNA. The results indicate that CD100 mRNA is expressed in many non-hematopoietic tissues including the heart, brain, placenta, lung, skeletal muscle, kidney and pancreas, but not the liver. Interestingly, the four major transcripts of 3.8, 4.5, 7 kb, and 10 kb are differentially expressed with a distinct tissue distribution of transcripts. In most of these tissues, the major transcript was the 4.5kb species. Heart and muscle express essentially the 3.8kb transcript. This transcript was also found to be expressed in brain, together with the other major transcripts.

Example 3: CD100 is primarily expressed in activated B cells within the lymphoid follicle germinal center

In this example, the level of CD100 protein was measured in lymphoid tissues by immunohistochemistry.

Cryostat sections of lymphoid tissue were fixed in acetone for ten minutes, washed with PBS and incubated for one hour with the following primary antibodies: a mouse monoclonal antibody against CD100, a mouse monoclonal antibody against CD20, a pan-B cell marker, and a monoclonal antibody against CD3, a pan-T cell marker. The sections were then washed with PBS, incubated with biotinylated horse anti-mouse antibody (Vector Laboratories, Burlingame, CA) for 30 minutes, washed with PBS, incubated with avidin:biotinylated-peroxidase complex (Vector Laboratories, Burlingame, CA) for 40 minutes, and reacted with diaminobenzidine/hydrogen peroxide. Sections were subsequently stained with 2% methyl green.

The results show that anti-CD100 mAb staining is primarily in the germinal center (GC), sporadic in the interfollicular areas and virtually absent in the mantle zone (MZ). This pattern differs from antibodies which stain with a dendritic cell pattern (e.g. CD23) and is consistent with B cell surface staining. On the contrary, a mAb against the B cell restricted molecule. CD20, stains most cells in both the GC and the MZ and a mAb against the T cell restricted molecule, CD3, stains cells in the interfollicular areas but very few cells in the GC and MZ. For comparison, a lymphoid tissue section was fixed in 10% buffered formaldehyde, paraffin embedded, sectioned and stained with hematoxylin and eosin. This section shows a germinal center and mantle zone of a secondary lymphoid follicle with interfollicular T cell zone. Thus, the results of this experiment indicate that the major hematopoietic cell type that encounters CD100 in the GC is the B lymphocyte. Furthermore, given the paucity of T cells in this lymphoid compartment, the major source of CD100 in the GC is likely to be activated B cells.

Thus, consistent with the high levels of CD100 mRNA expression in activated B cells, anti-CD100 mAb predominantly stained activated B cells within the lymphoid follicle germinal center.

Example 4: CD100 stimulates B cell homotypic aggregation and synergizes with CD40L

The effect of CD100 on B cells, was analyzed in an *in vitro* model system using stable CD100 transfectants (t-CD100). NIH-3T3 cells were mock transfected, transfected with CD100 cDNA or with CD40L cDNA. Transfected cells were harvested, irradiated (96Gy), plated at a fixed concentration of 10^5 total transfectants/well and incubated overnight. Human splenic B cells were cultured on the different transfectants at 10^6 B cells per 1 ml culture in 24-well plates in B cell medium (Carriere D et al., (1989), *Exp. Cell. Res.* 182, 114). Photographs of cell cultures were taken at a magnification of 100X at 72 hrs.

As shown in Figure 6d, the results of this example indicate that co-culture of human tonsillar B cells with irradiated t-CD100 stimulates the B cells to homotypically aggregate within 24 hours and form continually increasing clusters that are maximal in size by day 3. In contrast, no significant clusters were observed in the mock transfected controls (Figure 6a). Other B cell sources including splenic B cells, follicular lymphoma, and acute lymphoblastic leukemias are similarly induced to cluster by co-culture with t-CD100. Figure 6b, indicates that CD40L transfectants (t-CD40L) also induce B cells to aggregate and form clusters. To examine the outcome of combining CD40L and CD100 signals, B cells were cultured on equal numbers of t-CD100 and t-CD40L, i.e., 50% t-CD100/50% t-CD40L (hereinafter referred to as t-CD100/t-CD40L). The total number of transfectants was constant (i.e., identical to number plated in the t-mock, t-CD40L, or t-CD100 wells). Figure 6f shows that the combination of CD100 and CD40L signaling synergizes to give rise to extraordinarily large B cell clusters that were larger than those observed with either transfectant alone or with the control. Other ratios of transfectants were also examined (75% t-CD100/25% t-CD40L and 25% t-CD100/75% t-CD40L) and these consistently induce larger clusters than observed with t-CD40L or t-CD100 alone, or the equivalent t-mock/t-CD40L control. Taken together, these results demonstrate that CD100 can directly induce B cell cluster formation, moreover, and that, when the CD100 and CD40L signals are combined, cluster formation is markedly enhanced.

The adhesion molecules LFA-3, LFA-1 and ICAM-1 are not upregulated by t-CD100 or t-mock whereas t-CD40L upregulates these adhesion molecules on human B cells (Carriere D et al., (1989), *Exp. Cell. Res.* 182, 114). Furthermore, these adhesion molecules were similarly upregulated on tonsillar B cells following culture on t-mock/t-CD40L or t-CD100/t-CD40L. Thus, these results indicate that the CD100 induced B cell aggregation is not mediated by an increase in LFA-3, LFA-1, or ICAM-1. However, these

results do not exclude the possibility that other adhesion molecules might be responsible for this observation or alternatively that cluster formation results form the induction of conformational changes in adhesion molecules (Chan B.M. et al, (1991), *J. Immunol.* 147, 398).

Example 5: CD100 alone or with CD40L stimulates an increase in B cell survival

To determine whether t-CD100/t-CD40L had an effect on B cell survival and/or proliferation, the number of viable B cells was determined by Trypan blue exclusion analysis after 3 days of co-culture according to the conditions described above. As shown in Fig. 7, co-culture with t-mock cells resulted in significant numbers of B cells dying during the culture period. Co-culture with t-CD100 increased the number of viable cells compared to t-mock, but there were consistently fewer cells than observed with t-CD40L. t-CD100/t-CD40L stimulation consistently resulted in significantly greater numbers of B cells than the t-mock/t-CD40L control.

To further distinguish between viability and cellular proliferation, ^{3}H-thymidine incorporation was also assessed on day 3. As expected, t-CD40L induced significant B cell proliferation, whereas neither t-mock nor t-CD100 induced significant proliferation. Proliferation induced by the combination of t-CD100/t-CD40L was not significantly greater than that observed with t-CD40L/t-mock demonstrating that the effect of CD100 signaling is likely to be on viability rather than on proliferation.

Example 6: CD100 stimulation of B cells reduces CD40L induced increase in CD23 expression

This example shows that in addition to synergizing with CD40L in inducing B cell clustering, CD100 modifies CD40L signaling by reducing CD23 induction induced by CD40L.

A two-color FACS analysis of human splenic B cells was performed after 72 hours of co-culture with t-mock/t-CD40L cells or t-CD100/t-CD40L. Cells were stained with CD19 conjugated to phycoerythrin and CD23 conjugated to fluorescein isothiocyanate (Coulter, Hialeah, FL). Non-viable cells were excluded by propidium iodide counterstaining prior to analysis.

As shown in Figure 8, the percentage of CD23+CD19+ cells is consistently reduced (50-90% reduction) when the CD40L signal is delivered in the presence of CD100. In contrast, CD100 does not modify the induction of B7 family costimulatory molecules. These results indicate that the CD100 signal not only amplifies the CD40L viability signal but also modifies the CD40L differentiative signal and therefore is likely to be important for the generation, organization, and/or regulation of B cells within the germinal center.

Example 7: CD100 stimulates T cell proliferation

The previous examples demonstrated that CD100 stimulating B cells to aggregate, extends their lifespan, and reduces the expression of CD23. This example demonstrates that CD100 can also provide a costimulatory signal to T cells.

5×10^4 CD4+ T cells were cultured in media, with an anti-CD3 antibody at 1μg/ml, with an anti-CD3 antibody at 1 μg/ml and 2×10^4 NIH-3T3 cells transfected with B7-1 (t-B7-1), with an anti-CD3 antibody at 1 μg/ml and 2×10^4 NIH-3T3 cells transfected with B7-2 (t-B7-2), or with an anti-CD3 antibody at 1 μg/ml and 2×10^4 t-CD100 cells. ^{3}H-thymidine incorporation was assessed on day 3.

The results are shown in Figure 9. The results indicate that, similarly to t-B7-1 and t-B7-2, t-CD100 stimulate the proliferation of CD4+ T cells. Thus, these results indicate that CD100 is capable of stimulating the proliferation of T cells in the presence of a primary activation signal.

In another example the costimulatory effect of various amounts of t-CD100 cells on CD4+ T cells was assessed. 5×10^4 CD4+ T cells were incubated with 1 μg/ml anti-CD3 antibody, 2×10^4 t-B7-1 cells and 0, 1×10^4, 2×10^4, or 3×10^4 t-CD100 cells or t-B7-1 cells. ^{3}H-thymidine incorporation was assessed on day 3. The results, which are presented in Figure 10, indicate that the level of T cell proliferation increases progressively with increasing amounts of t-CD100 cells. The level of T cell proliferation obtained by incubating T cells with a mixture of t-B7-1 and t-CD100 cells is similar to the level of T cell proliferation obtained by incubating T cells with t-CD100.

In a similar experiment, 5×10^4 CD4+ T cells were incubated with 1 μg/ml anti-CD3 antibody, 2×10^4 t-B7-2 cells and 0, 1×10^4, 2×10^4, or 3×10^4 t-CD100 cells or t-B7-2 cells and thymidine incorporation was determined after 3 days of culture. The results, shown in Figure 11, indicate that T cell proliferation increases progressively with increasing amounts of t-CD100 and t-B7-2 cells.

Thus, CD100 is capable of stimulating T cells to proliferate in the presence of a primary activation signal.

Example 8: Cloning of a cDNA encoding murine CD100 and characterization of murine CD100

This Example describes the isolation of a cDNA encoding a murine CD100 (mCD100) molecule and characteristics of mouse CD100 protein.

A cDNA library prepared from murine T cells activated with anti-CD3 and anti-CD28 antibodies was screened with a probe corresponding to a nucleic acid encoding the sema and Ig domains of the human CD100 protein. The hybridization was carried out at 60° C under low stringency conditions (in the absence of formamide) with a final wash at 55° C in 2 x SSC, 0.1% SDS. More than 40 cDNA clones were obtained. One of these

clones contained an insert having the nucleic acid sequence SEQ ID NO: 8 shown in Figure 12 which encodes the full length mouse CD100 protein.

Northern blot hybridization using mCD100 cDNA indicated the presence of a 5.1 kb murine CD100 RNA in the Balb/c B lymphoma cell line A20. No mCD100 RNA was detected in L929 fibroblast cells and Chinese Hamster Ovary (CHO) cells.

Southern blot analysis of mouse genomic DNA with mCD100 cDNA indicated that the exons of the mCD100 gene are compactly organized. Only a single major band was evident after HindIII digestion along with 4 minor bands that might be small exons or other semaphorin gene family members. Three bands were observed after BamHI or EcoRI digestion.

Nucleic acid and amino acid sequence comparisons indicated that human and mouse CD100 are highly similar. Figure 13 shows a nucleic acid sequence comparison between mouse CD100 cDNA (SEQ ID NO: 8) and the open reading frame of human CD100 (SEQ ID NO: 22). This comparison shows that the open reading frame of human and mouse CD100 cDNA have 2141 out of 2586 nucleotides that are identical. Thus, mouse and human CD100 open reading frame nucleotide sequences have 83% homology or similarity.

A comparison between the amino acid sequence of human and mouse CD100 proteins indicated that the structural features of mouse CD100 (SEQ ID NO: 10) are similar to those of human CD100 protein (SEQ ID NO: 2). Similarly to human CD100, mouse CD100 protein has a signal, sema, Ig, stalk, transmembrane, and cytoplasmic domain, which are all highly conserved between mouse and human CD100. Mouse CD100 protein has a signal sequence from about amino acid 1 to about amino acid 22 or 24 of amino acid sequence shown in Figure 16 (SEQ ID NO: 9), a sema domain from about amino acid 42 to about amino acid 553 of amino acid sequence shown in Figure 14 (SEQ ID NO: 9), an Ig domain from about amino acid 554 or 566 to about amino acid 630 of amino acid sequence shown in Figure 14 (SEQ ID NO: 9), a stalk domain from about amino acid 631 to about amino acid 732 of amino acid sequence shown in Figure 14 (SEQ ID NO: 9), a transmembrane domain from about amino acid 734 to about amino acid 752 of amino acid sequence shown in Figure 14 (SEQ ID NO: 9), and a cytoplasmic domain from about amino acid 753 to about amino acid 861.

A comparison of the amino acid sequences of each domain of human and mouse CD100 proteins is shown in Figure 15 and the percentage homology between each domain is indicated in Table I. In this comparison, the domains are considered as having the following portions of the protein: signal peptide domain corresponding to amino acids 1-41 in mouse and human CD100; sema domain corresponding to amino acids 42-553 in mouse and human CD100; Ig-like domain corresponding to amino acids 554-630 in mouse and human CD100; stalk domain corresponding to amino acids 631-732 in mouse CD100 and amino acids 631-733 in human CD100; transmembrane domain corresponding to amino acids 734-752 in mouse CD100 and to amino acids 735-752 in human CD100; and

cytoplasmic domain corresponding to amino acids 753-861 in mouse CD100 and amino acids 754 to 862 in human CD100. The signal peptide, Sema domain, Ig domain, transmembrane (TM) domain and cytoplasmic domains of each protein have the same number of amino acids. The stalk domain of human CD100 is one amino acid (aa) longer than that of mouse CD100.

Table I
CD100, mouse/human comparison

Domain	# Identical aa	# Conserved aa	Total aa	% Identity
Signal	28	8	41	68%
Sema	454	19	512	88%
Ig	65	5	77	84%
Stalk	73	10	m103 or h104	70%
TM	16	1	19	84%
Cyto	102	4	109	94%
Total	738	47	m861 h862	86%

Table I indicates that among the protein domains, the cytoplasmic domain was most conserved, having 93% identity between the human and mouse domain. This is indicative of an important signaling function of the cytoplasmic (cyto) domain. The Sema domain is also highly conserved, having 454 amino acids out of 512 amino acids which are identical between mouse and human, corresponding to 88% identity. Most of the amino acid differences in the sema domain are in the COOH end. The stalk region tethering the Sema and Ig domains to the membrane was least conserved at 70% identity. Overall, mouse and human CD100 have 86% amino acid identity and 91% similarity (i.e., if conservative amino acid substitutions are allowed).

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Hall, Kathryn T. et al.

(ii) TITLE OF INVENTION: CD100 Antigen and Uses Therefor

(iii) NUMBER OF SEQUENCES: 22

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: LAHIVE & COCKFIELD
(B) STREET: 60 State Street, suite 510
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02109-1875

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/556,422
(B) FILING DATE: 09-NOV-1995

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Mandragouras, Amy E.
(B) REGISTRATION NUMBER: 36,207
(C) REFERENCE/DOCKET NUMBER: DFN-005CPPC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617)227-7400
(B) TELEFAX: (617)227-5941

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4157 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 88..2674

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CTGAGCCGCA TCTGCAATAG CACACTTGCC CGGCCACCTG CTGCCGTGAG CCTTTGCTGC      60

TGAAGCCCCT GGGGTCGCCT CTACCTG ATG AGG ATG TGC ACC CCC ATT AGG          111
                              Met Arg Met Cys Thr Pro Ile Arg
                                1               5

GGG CTG CTC ATG GCC CTT GCA GTG ATG TTT GGG ACA GCG ATG GCA TTT        159
Gly Leu Leu Met Ala Leu Ala Val Met Phe Gly Thr Ala Met Ala Phe
     10                  15                  20

GCA CCC ATA CCC CGG ATC ACC TGG GAG CAC AGA GAG GTG CAC CTG GTG        207
Ala Pro Ile Pro Arg Ile Thr Trp Glu His Arg Glu Val His Leu Val
 25                  30                  35                  40

CAG TTT CAT GAG CCA GAC ATC TAC AAC TAC TCA GCC TTG CTG CTG AGC        255
Gln Phe His Glu Pro Asp Ile Tyr Asn Tyr Ser Ala Leu Leu Leu Ser
                    45                  50                  55

GAG GAC AAG GAC ACC TTG TAC ATA GGT GCC CGG GAG GCG GTC TTC GCT        303
Glu Asp Lys Asp Thr Leu Tyr Ile Gly Ala Arg Glu Ala Val Phe Ala
               60                  65                  70

GTG AAC GCA CTC AAC ATC TCC GAG AAG CAG CAT GAG GTG TAT TGG AAG        351
Val Asn Ala Leu Asn Ile Ser Glu Lys Gln His Glu Val Tyr Trp Lys
          75                  80                  85

GTC TCA GAA GAC AAA AAA GCA AAA TGT GCA GAA AAG GGG AAA TCA AAA        399
Val Ser Glu Asp Lys Lys Ala Lys Cys Ala Glu Lys Gly Lys Ser Lys
     90                  95                  100

CAG ACA GAG TGC CTC AAC TAC ATC CGG GTG CTG CAG CCA CTC AGC GCC        447
Gln Thr Glu Cys Leu Asn Tyr Ile Arg Val Leu Gln Pro Leu Ser Ala
105                 110                 115                 120

ACT TCC CTT TAC GTG TGT GGG ACC AAC GCA TTC CAG CCG GCC TGT GAC        495
Thr Ser Leu Tyr Val Cys Gly Thr Asn Ala Phe Gln Pro Ala Cys Asp
                125                 130                 135

CAC CTG AAC TTA ACA TCC TTT AAG TTT CTG GGG AAA AAT GAA GAT GGC        543
His Leu Asn Leu Thr Ser Phe Lys Phe Leu Gly Lys Asn Glu Asp Gly
           140                 145                 150

AAA GGA AGA TGT CCC TTT GAC CCA GCA CAC AGC TAC ACA TCC GTC ATG        591
Lys Gly Arg Cys Pro Phe Asp Pro Ala His Ser Tyr Thr Ser Val Met
      155                 160                 165

GTT GAT GGA GAA CTT TAT TCG GGG ACG TCG TAT AAT TTT TTG GGA AGT        639
Val Asp Gly Glu Leu Tyr Ser Gly Thr Ser Tyr Asn Phe Leu Gly Ser
 170                 175                 180

GAA CCC ATC ATC TCC CGA AAT TCT TCC CAC AGT CCT CTG AGG ACA GAA        687
Glu Pro Ile Ile Ser Arg Asn Ser Ser His Ser Pro Leu Arg Thr Glu
185                 190                 195                 200
```

```
TAT GCA ATC CCT TGG CTG AAC GAG CCT AGT TTC GTG TTT GCT GAC GTG      735
Tyr Ala Ile Pro Trp Leu Asn Glu Pro Ser Phe Val Phe Ala Asp Val
                205                 210                 215

ATC CGA AAA AGC CCA GAC AGC CCC GAC GGC GAG GAT GAC AGG GTC TAC      783
Ile Arg Lys Ser Pro Asp Ser Pro Asp Gly Glu Asp Asp Arg Val Tyr
            220                 225                 230

TTC TTC TTC ACG GAG GTG TCT GTG GAG TAT GAG TTT GTG TTC AGG GTG      831
Phe Phe Phe Thr Glu Val Ser Val Glu Tyr Glu Phe Val Phe Arg Val
        235                 240                 245

CTG ATC CCA CGG ATA GCA AGA GTG TGC AAG GGG GAC CAG GGC GGC CTG      879
Leu Ile Pro Arg Ile Ala Arg Val Cys Lys Gly Asp Gln Gly Gly Leu
    250                 255                 260

AGG ACC TTG CAG AAG AAA TGG ACC TCC TTC CTG AAA GCC CGA CTC ATC      927
Arg Thr Leu Gln Lys Lys Trp Thr Ser Phe Leu Lys Ala Arg Leu Ile
265                 270                 275                 280

TGC TCC CGG CCA GAC AGC GGC TTG GTC TTC AAT GTG CTG CGG GAT GTC      975
Cys Ser Arg Pro Asp Ser Gly Leu Val Phe Asn Val Leu Arg Asp Val
                285                 290                 295

TTC GTG CTC AGG TCC CCG GGC CTG AAG GTG CCT GTG TTC TAT GCA CTC     1023
Phe Val Leu Arg Ser Pro Gly Leu Lys Val Pro Val Phe Tyr Ala Leu
            300                 305                 310

TTC ACC CCA CAG CTG AAC AAC GTG GGG CTG TCG GCA GTG TGC GCC TAC     1071
Phe Thr Pro Gln Leu Asn Asn Val Gly Leu Ser Ala Val Cys Ala Tyr
        315                 320                 325

AAC CTG TCC ACA GCC GAG GAG GTC TTC TCC CAC GGG AAG TAC ATG CAG     1119
Asn Leu Ser Thr Ala Glu Glu Val Phe Ser His Gly Lys Tyr Met Gln
    330                 335                 340

AGC ACC ACA GTG GAG CAG TCC CAC ACC AAG TGG GTG CGC TAT AAT GGC     1167
Ser Thr Thr Val Glu Gln Ser His Thr Lys Trp Val Arg Tyr Asn Gly
345                 350                 355                 360

CCG GTA CCC AAG CCG CGG CCT GGA GCG TGC ATC GAC AGC GAG GCA CGG     1215
Pro Val Pro Lys Pro Arg Pro Gly Ala Cys Ile Asp Ser Glu Ala Arg
                365                 370                 375

GCC GCC AAC TAC ACC AGC TCC TTG AAT TTG CCA GAC AAG ACG CTG CAG     1263
Ala Ala Asn Tyr Thr Ser Ser Leu Asn Leu Pro Asp Lys Thr Leu Gln
            380                 385                 390

TTC GTT AAA GAC CAC CCT TTG ATG GAT GAC TCG GTA ACC CCA ATA GAC     1311
Phe Val Lys Asp His Pro Leu Met Asp Asp Ser Val Thr Pro Ile Asp
        395                 400                 405

AAC AGG CCC AGG TTA ATC AAG AAA GAT GTG AAC TAC ACC CAG ATC GTG     1359
Asn Arg Pro Arg Leu Ile Lys Lys Asp Val Asn Tyr Thr Gln Ile Val
    410                 415                 420
```

```
GTG GAC CGG ACC CAG GCC CTG GAT GGG ACT GTC TAT GAT GTC ATG TTT      1407
Val Asp Arg Thr Gln Ala Leu Asp Gly Thr Val Tyr Asp Val Met Phe
425                 430                 435                 440

GTC AGC ACA GAC CGG GGA GCT CTG CAC AAA GCC ATC AGC CTC GAG CAC      1455
Val Ser Thr Asp Arg Gly Ala Leu His Lys Ala Ile Ser Leu Glu His
                445                 450                 455

GCT GTT CAC ATC ATC GAG GAG ACC CAG CTC TTC CAG GAC TTT GAG CCA      1503
Ala Val His Ile Ile Glu Glu Thr Gln Leu Phe Gln Asp Phe Glu Pro
            460                 465                 470

GTC CAG ACC CTG CTG CTG TCT TCA AAG AAG GGC AAC AGG TTT GTC TAT      1551
Val Gln Thr Leu Leu Leu Ser Ser Lys Lys Gly Asn Arg Phe Val Tyr
        475                 480                 485

GCT GGC TCT AAC TCG GGC GTG GTC CAG GCC CCG CTG GCC TTC TGT GGG      1599
Ala Gly Ser Asn Ser Gly Val Val Gln Ala Pro Leu Ala Phe Cys Gly
    490                 495                 500

AAG CAC GGC ACC TGC GAG GAC TGT GTG CTG GCG CGG GAC CCC TAC TGC      1647
Lys His Gly Thr Cys Glu Asp Cys Val Leu Ala Arg Asp Pro Tyr Cys
505                 510                 515                 520

GCC TGG AGC CCG CCC ACA GCG ACC TGC GTG GCT CTG CAC CAG ACC GAG      1695
Ala Trp Ser Pro Pro Thr Ala Thr Cys Val Ala Leu His Gln Thr Glu
                525                 530                 535

AGC CCC AGC AGG GGT TTG ATT CAG GAG ATG AGC GGC GAT GCT TCT GTG      1743
Ser Pro Ser Arg Gly Leu Ile Gln Glu Met Ser Gly Asp Ala Ser Val
            540                 545                 550

TGC CCG GAT AAA AGT AAA GGA AGT TAC CGG CAG CAT TTT TTC AAG CAC      1791
Cys Pro Asp Lys Ser Lys Gly Ser Tyr Arg Gln His Phe Phe Lys His
        555                 560                 565

GGT GGC ACA GCG GAA CTG AAA TGC TCC CAA AAA TCC AAC CTG GCC CGG      1839
Gly Gly Thr Ala Glu Leu Lys Cys Ser Gln Lys Ser Asn Leu Ala Arg
    570                 575                 580

GTC TTT TGG AAG TTC CAG AAT GGC GTG TTG AAG GCC GAG AGC CCC AAG      1887
Val Phe Trp Lys Phe Gln Asn Gly Val Leu Lys Ala Glu Ser Pro Lys
585                 590                 595                 600

TAC GGT CTT ATG GGC AGA AAA AAC TTG CTC ATC TTC AAC TTG TCA GAA      1935
Tyr Gly Leu Met Gly Arg Lys Asn Leu Leu Ile Phe Asn Leu Ser Glu
                605                 610                 615

GGA GAC AGT GGG GTG TAC CAG TGC CTG TCA GAG GAG AGG GTT AAG AAC      1983
Gly Asp Ser Gly Val Tyr Gln Cys Leu Ser Glu Glu Arg Val Lys Asn
            620                 625                 630

AAA ACG GTC TTC CAA GTG GTC GCC AAG CAC GTC CTG GAA GTG AAG GTG      2031
Lys Thr Val Phe Gln Val Val Ala Lys His Val Leu Glu Val Lys Val
        635                 640                 645
```

```
GTT CCA AAG CCC GTA GTG GCC CCC ACC TTG TCA GTT GTT CAG ACA GAA      2079
Val Pro Lys Pro Val Val Ala Pro Thr Leu Ser Val Val Gln Thr Glu
    650                 655                 660

GGT AGT AGG ATT GCC ACC AAA GTG TTG GTG GCA TCC ACC CAA GGG TCT      2127
Gly Ser Arg Ile Ala Thr Lys Val Leu Val Ala Ser Thr Gln Gly Ser
665                 670                 675                 680

TCT CCC CCA ACC CCA GCC GTG CAG GCC ACC TCC TCC GGG GCC ATC ACC      2175
Ser Pro Pro Thr Pro Ala Val Gln Ala Thr Ser Ser Gly Ala Ile Thr
                685                 690                 695

CTT CCT CCC AAG CCT GCG CCC ACC GGC ACA TCC TGC GAA CCA AAG ATC      2223
Leu Pro Pro Lys Pro Ala Pro Thr Gly Thr Ser Cys Glu Pro Lys Ile
            700                 705                 710

GTC ATC AAC ACG GTC CCC CAG CTC CAC TCG GAG AAA ACC ATG TAT CTT      2271
Val Ile Asn Thr Val Pro Gln Leu His Ser Glu Lys Thr Met Tyr Leu
        715                 720                 725

AAG TCC AGC GAC AAC CGC CTC CTC ATG TCC CTC TTC CTC TTC TTC TTT      2319
Lys Ser Ser Asp Asn Arg Leu Leu Met Ser Leu Phe Leu Phe Phe Phe
    730                 735                 740

GTT CTC TTC CTC TGC CTC TTT TTC TAC AAC TGC TAT AAG GGA TAC CTG      2367
Val Leu Phe Leu Cys Leu Phe Phe Tyr Asn Cys Tyr Lys Gly Tyr Leu
745                 750                 755                 760

CCC AGA CAG TGC TTG AAA TTC CGC TCG GCC CTA CTA ATT GGG AAG AAG      2415
Pro Arg Gln Cys Leu Lys Phe Arg Ser Ala Leu Leu Ile Gly Lys Lys
                765                 770                 775

AAG CCC AAG TCA GAT TTC TGT GAC CGT GAG CAG AGC CTG AAG GAG ACG      2463
Lys Pro Lys Ser Asp Phe Cys Asp Arg Glu Gln Ser Leu Lys Glu Thr
            780                 785                 790

TTA GTA GAG CCA GGG AGC TTC TCC CAG CAG AAT GGG GAG CAC CCC AAG      2511
Leu Val Glu Pro Gly Ser Phe Ser Gln Gln Asn Gly Glu His Pro Lys
        795                 800                 805

CCA GCC CTG GAC ACC GGC TAT GAG ACC GAG CAA GAC ACC ATC ACC AGC      2559
Pro Ala Leu Asp Thr Gly Tyr Glu Thr Glu Gln Asp Thr Ile Thr Ser
    810                 815                 820

AAA GTC CCC ACG GAT AGG GAG GAC TCA CAG AGG ATC GAC GAC CTT TCT      2607
Lys Val Pro Thr Asp Arg Glu Asp Ser Gln Arg Ile Asp Asp Leu Ser
825                 830                 835                 840

GCC AGG GAC AAG CCC TTT GAC GTC AAG TGT GAG CTG AAG TTC GCT GAC      2655
Ala Arg Asp Lys Pro Phe Asp Val Lys Cys Glu Leu Lys Phe Ala Asp
                845                 850                 855

TCA GAC GCA GAT GGA GAC T GAGGCCGGCT GTGCATCCCC GCTGGTGCCT            2704
Ser Asp Ala Asp Gly Asp  *
            860

CGGCTGCGAC GTGTCCAGGC GTGGAGAGTT TTGTGTTTCT CCTGTTCAGT ATCCGAGTCT    2764
```

CGTGCAGTGC TGCGTAGGTT AGCCCGCATC GTGCAGACAA CCTCAGTCCT CTTGTCTATT 2824

TTCTCTTGGG TTGAGCCTGT GACTTGGTTT CTCTTTGTCC TTTTGGAAAA ATGACAAGCA 2884

TTGCATCCCA GTCTTGTGTT CCGAAGTCAG TCGGAGTACT TGAAGAAGGC CCACGGGCGG 2944

CACGGAGTTC CTGAGCCCTT TCTGTAGTGG GGGAAAGGTG GCTGGACCTC TGTTGGCTGA 3004

GAAGAGCATC CCTTCAGCTT CCCCTCCCCG TAGCAGCCAC TAAAAGATTA TTTAATTCCA 3064

GATTGGAAAT GACATTTTAG TTTATCAGAT TGGTAACTTA TCGCCTGTTG TCCAGATTGG 3124

CACGAACCTT TTCTTCCACT TAATTATTTT TTTAGGATTT TGCTTTGATT GTGTTTATGT 3184

CATGGGTCAT TTTTTTTTAG TTACAGAAGC AGTTGTGTTA ATATTTAGAA GAAGATGTAT 3244

ATCTTCCAGA TTTTGTTATA TATTTGGCAT AAAATACGGC TTACGTTGCT TAAGATTCTC 3304

AGGGATAAAC TTCCTTTTGC TAAATGCATT CTTTCTGCTT TTAGAAATGT AGACATAAAC 3364

ACTCCCCGGA GCCCACTCAC CTTTTTTCTT TTTCTTTTTT TTTTTTTAAC TTTATTCCTT 3424

GAGGGAAGCA TTGTTTTTGG AGAGATTTTC TTTCTGTACT TCGTTTTACT TTTCTTTTTT 3484

TTTAACTTTT ACTCTCTCGA AGAAGAGGAC CTTCCCACAT CCACGAGGTG GGTTTTGAGC 3544

AAGGGAAGGT AGCCTGGATG AGCTGAGTGG AGCCAGGCTG GCCCAGAGCT GAGATGGGAG 3604

TGCGGTACAA TCTGGAGCCC ACAGCTGTCG GTCAGAACCT CCTGTGAGAC AGATGGAACC 3664

TTCACAAGGG CGCCTTTGGT TCTCTGAACA TCTCCTTTCT CTTCTTGCTT CAATTGCTTA 3724

CCCACTGCCT GCCCAGACTT TCTATCCAGC CTCACTGAGC TGCCCACTAC TGGAAGGGAA 3784

CTGGGCCTCG GTGGCCGGGG CCGCGAGCTG TGACCACAGC ACCCTCAAGC ATACGGCGCT 3844

GTTCCTGCCA CTGTCCTGAA GATGTGAATG GGTGGTACGA TTTCAACACT GGTTAATTTC 3904

ACACTCCATC TCCCCGCTTT GTAAATACCC ATCGGGAAGA GACTTTTTTT CCATGGTGAA 3964

GAGCAATAAA CTCTGGATGT TTGTGCGCGT GTGTGGACAG TCTTATCTTC CAGCATGATA 4024

GGATTTGACC ATTTTGGTGT AAACATTTGT GTTTTATAAG ATTTACCTTG TTTTTATTTT 4084

TCTACTTTGA ATTGTATACA TTTGGAAAGT ACCCAAATAA ATGAGAAGCT TCTATCCTTA 4144

AAAAAAAAAA AAA 4157

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 862 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Arg Met Cys Thr Pro Ile Arg Gly Leu Leu Met Ala Leu Ala Val
1 5 10 15

Met Phe Gly Thr Ala Met Ala Phe Ala Pro Ile Pro Arg Ile Thr Trp
20 25 30

Glu His Arg Glu Val His Leu Val Gln Phe His Glu Pro Asp Ile Tyr
35 40 45

Asn Tyr Ser Ala Leu Leu Leu Ser Glu Asp Lys Asp Thr Leu Tyr Ile
50 55 60

Gly Ala Arg Glu Ala Val Phe Ala Val Asn Ala Leu Asn Ile Ser Glu
65 70 75 80

Lys Gln His Glu Val Tyr Trp Lys Val Ser Glu Asp Lys Lys Ala Lys
85 90 95

Cys Ala Glu Lys Gly Lys Ser Lys Gln Thr Glu Cys Leu Asn Tyr Ile
100 105 110

Arg Val Leu Gln Pro Leu Ser Ala Thr Ser Leu Tyr Val Cys Gly Thr
115 120 125

Asn Ala Phe Gln Pro Ala Cys Asp His Leu Asn Leu Thr Ser Phe Lys
130 135 140

Phe Leu Gly Lys Asn Glu Asp Gly Lys Gly Arg Cys Pro Phe Asp Pro
145 150 155 160

Ala His Ser Tyr Thr Ser Val Met Val Asp Gly Glu Leu Tyr Ser Gly
165 170 175

Thr Ser Tyr Asn Phe Leu Gly Ser Glu Pro Ile Ile Ser Arg Asn Ser
180 185 190

Ser His Ser Pro Leu Arg Thr Glu Tyr Ala Ile Pro Trp Leu Asn Glu
195 200 205

Pro Ser Phe Val Phe Ala Asp Val Ile Arg Lys Ser Pro Asp Ser Pro
210 215 220

Asp Gly Glu Asp Asp Arg Val Tyr Phe Phe Phe Thr Glu Val Ser Val
225 230 235 240

Glu Tyr Glu Phe Val Phe Arg Val Leu Ile Pro Arg Ile Ala Arg Val
245 250 255

Cys Lys Gly Asp Gln Gly Gly Leu Arg Thr Leu Gln Lys Lys Trp Thr
260 265 270

Ser Phe Leu Lys Ala Arg Leu Ile Cys Ser Arg Pro Asp Ser Gly Leu
275 280 285

Val Phe Asn Val Leu Arg Asp Val Phe Val Leu Arg Ser Pro Gly Leu
290 295 300

```
Lys Val Pro Val Phe Tyr Ala Leu Phe Thr Pro Gln Leu Asn Asn Val
305                 310                 315                 320

Gly Leu Ser Ala Val Cys Ala Tyr Asn Leu Ser Thr Ala Glu Glu Val
                325                 330                 335

Phe Ser His Gly Lys Tyr Met Gln Ser Thr Thr Val Glu Gln Ser His
            340                 345                 350

Thr Lys Trp Val Arg Tyr Asn Gly Pro Val Pro Lys Pro Arg Pro Gly
        355                 360                 365

Ala Cys Ile Asp Ser Glu Ala Arg Ala Ala Asn Tyr Thr Ser Ser Leu
    370                 375                 380

Asn Leu Pro Asp Lys Thr Leu Gln Phe Val Lys Asp His Pro Leu Met
385                 390                 395                 400

Asp Asp Ser Val Thr Pro Ile Asp Asn Arg Pro Arg Leu Ile Lys Lys
                405                 410                 415

Asp Val Asn Tyr Thr Gln Ile Val Val Asp Arg Thr Gln Ala Leu Asp
            420                 425                 430

Gly Thr Val Tyr Asp Val Met Phe Val Ser Thr Asp Arg Gly Ala Leu
        435                 440                 445

His Lys Ala Ile Ser Leu Glu His Ala Val His Ile Ile Glu Glu Thr
    450                 455                 460

Gln Leu Phe Gln Asp Phe Glu Pro Val Gln Thr Leu Leu Leu Ser Ser
465                 470                 475                 480

Lys Lys Gly Asn Arg Phe Val Tyr Ala Gly Ser Asn Ser Gly Val Val
                485                 490                 495

Gln Ala Pro Leu Ala Phe Cys Gly Lys His Gly Thr Cys Glu Asp Cys
            500                 505                 510

Val Leu Ala Arg Asp Pro Tyr Cys Ala Trp Ser Pro Pro Thr Ala Thr
        515                 520                 525

Cys Val Ala Leu His Gln Thr Glu Ser Pro Ser Arg Gly Leu Ile Gln
    530                 535                 540

Glu Met Ser Gly Asp Ala Ser Val Cys Pro Asp Lys Ser Lys Gly Ser
545                 550                 555                 560

Tyr Arg Gln His Phe Phe Lys His Gly Gly Thr Ala Glu Leu Lys Cys
                565                 570                 575

Ser Gln Lys Ser Asn Leu Ala Arg Val Phe Trp Lys Phe Gln Asn Gly
            580                 585                 590

Val Leu Lys Ala Glu Ser Pro Lys Tyr Gly Leu Met Gly Arg Lys Asn
        595                 600                 605
```

Leu Leu Ile Phe Asn Leu Ser Glu Gly Asp Ser Gly Val Tyr Gln Cys
610 615 620

Leu Ser Glu Glu Arg Val Lys Asn Lys Thr Val Phe Gln Val Val Ala
625 630 635 640

Lys His Val Leu Glu Val Lys Val Val Pro Lys Pro Val Val Ala Pro
645 650 655

Thr Leu Ser Val Val Gln Thr Glu Gly Ser Arg Ile Ala Thr Lys Val
660 665 670

Leu Val Ala Ser Thr Gln Gly Ser Ser Pro Pro Thr Pro Ala Val Gln
675 680 685

Ala Thr Ser Ser Gly Ala Ile Thr Leu Pro Pro Lys Pro Ala Pro Thr
690 695 700

Gly Thr Ser Cys Glu Pro Lys Ile Val Ile Asn Thr Val Pro Gln Leu
705 710 715 720

His Ser Glu Lys Thr Met Tyr Leu Lys Ser Ser Asp Asn Arg Leu Leu
725 730 735

Met Ser Leu Phe Leu Phe Phe Phe Val Leu Phe Leu Cys Leu Phe Phe
740 745 750

Tyr Asn Cys Tyr Lys Gly Tyr Leu Pro Arg Gln Cys Leu Lys Phe Arg
755 760 765

Ser Ala Leu Leu Ile Gly Lys Lys Lys Pro Lys Ser Asp Phe Cys Asp
770 775 780

Arg Glu Gln Ser Leu Lys Glu Thr Leu Val Glu Pro Gly Ser Phe Ser
785 790 795 800

Gln Gln Asn Gly Glu His Pro Lys Pro Ala Leu Asp Thr Gly Tyr Glu
805 810 815

Thr Glu Gln Asp Thr Ile Thr Ser Lys Val Pro Thr Asp Arg Glu Asp
820 825 830

Ser Gln Arg Ile Asp Asp Leu Ser Ala Arg Asp Lys Pro Phe Asp Val
835 840 845

Lys Cys Glu Leu Lys Phe Ala Asp Ser Asp Ala Asp Gly Asp
850 855 860

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 655 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Gly Trp Leu Thr Arg Ile Val Cys Leu Phe Trp Gly Val Leu Leu
1               5                  10                  15

Thr Ala Arg Ala Asn Tyr Gln Asn Gly Lys Asn Asn Val Pro Arg Leu
            20                  25                  30

Lys Leu Ser Tyr Lys Glu Met Leu Glu Ser Asn Asn Val Ile Thr Phe
        35                  40                  45

Asn Gly Leu Ala Asn Ser Ser Ser Tyr His Thr Phe Leu Leu Asp Glu
    50                  55                  60

Glu Arg Ser Arg Leu Tyr Val Gly Ala Lys Asp His Ile Phe Ser Phe
65                  70                  75                  80

Asp Leu Val Asn Ile Lys Asp Phe Gln Lys Ile Val Trp Pro Val Ser
                85                  90                  95

Tyr Thr Arg Arg Asp Glu Cys Lys Trp Ala Gly Lys Asp Ile Leu Lys
            100                 105                 110

Glu Cys Ala Asn Phe Ile Lys Val Leu Lys Ala Tyr Asn Gln Thr His
        115                 120                 125

Leu Tyr Ala Cys Gly Thr Gly Ala Phe His Pro Ile Cys Thr Tyr Ile
    130                 135                 140

Glu Ile Gly His His Pro Glu Asp Asn Ile Phe Lys Leu Glu Asn Ser
145                 150                 155                 160

His Phe Glu Asn Gly Arg Gly Lys Ser Pro Tyr Asp Pro Lys Leu Leu
                165                 170                 175

Thr Ala Ser Leu Leu Ile Asp Gly Glu Leu Tyr Ser Gly Thr Ala Ala
            180                 185                 190

Asp Phe Met Gly Arg Asp Phe Ala Ile Phe Arg Thr Leu Gly His His
        195                 200                 205

His Pro Ile Arg Thr Glu Gln His Asp Ser Arg Trp Leu Asn Asp Pro
    210                 215                 220

Lys Phe Ile Ser Ala His Leu Ile Ser Glu Ser Asp Asn Pro Glu Asp
225                 230                 235                 240

Asp Lys Val Tyr Phe Phe Phe Arg Glu Asn Ala Ile Asp Gly Glu His
                245                 250                 255

Ser Gly Lys Ala Thr His Ala Arg Ile Gly Gln Ile Cys Lys Asn Asp
            260                 265                 270
```

Phe Gly Gly His Arg Ser Leu Val Asn Lys Trp Thr Thr Phe Leu Lys
275 280 285

Ala Arg Leu Ile Cys Ser Val Pro Gly Pro Asn Gly Ile Asp Thr His
290 295 300

Phe Asp Glu Leu Gln Asp Val Phe Leu Met Asn Phe Lys Asp Pro Lys
305 310 315 320

Asn Pro Val Val Tyr Gly Val Phe Thr Thr Ser Ser Asn Ile Phe Lys
325 330 335

Gly Ser Ala Val Cys Met Tyr Ser Met Ser Asp Val Arg Arg Val Phe
340 345 350

Leu Gly Pro Tyr Ala His Arg Asp Gly Pro Asn Tyr Gln Trp Val Pro
355 360 365

Tyr Gln Gly Arg Val Pro Tyr Pro Arg Pro Gly Thr Cys Pro Ser Lys
370 375 380

Thr Phe Gly Gly Phe Asp Ser Thr Lys Asp Leu Pro Asp Asp Val Ile
385 390 395 400

Thr Phe Ala Arg Ser His Pro Ala Met Tyr Asn Pro Val Phe Pro Met
405 410 415

Asn Asn Arg Pro Ile Val Ile Lys Thr Asp Val Asn Tyr Gln Phe Thr
420 425 430

Gln Ile Val Val Asp Arg Val Asp Ala Glu Asp Gly Gln Tyr Asp Val
435 440 445

Met Phe Ile Gly Thr Asp Val Gly Thr Val Leu Lys Val Val Ser Ile
450 455 460

Pro Lys Glu Thr Trp Tyr Asp Leu Glu Glu Val Leu Leu Glu Glu Met
465 470 475 480

Thr Val Phe Arg Glu Pro Thr Ala Ile Ser Ala Met Glu Leu Ser Thr
485 490 495

Lys Gln Gln Gln Leu Tyr Ile Gly Ser Thr Ala Gly Val Ala Gln Leu
500 505 510

Pro Leu His Arg Cys Asp Ile Tyr Gly Lys Ala Cys Ala Glu Cys Cys
515 520 525

Leu Ala Arg Asp Pro Tyr Cys Ala Trp Asp Gly Ser Ala Cys Ser Arg
530 535 540

Tyr Phe Pro Thr Ala Lys Arg Arg Thr Arg Arg Gln Asp Ile Arg Asn
545 550 555 560

Gly Asp Pro Leu Thr His Cys Ser Asp Leu His His Asp Asn His His
565 570 575

Gly His Ser Pro Glu Glu Arg Ile Ile Tyr Gly Val Glu Asn Ser Ser
580 585 590

Thr Phe Leu Glu Cys Ser Pro Lys Ser Gln Arg Ala Leu Val Tyr Trp
595 600 605

Gln Phe Gln Arg Arg Asn Glu Glu Arg Lys Glu Glu Ile Arg Val Asp
610 615 620

Asp His Ile Ile Arg Thr Asp Gln Gly Leu Leu Leu Arg Ser Leu Gln
625 630 635 640

Gln Lys Asp Ser Gly Asn Tyr Leu Cys His Ala Val Glu His Gly
645 650 655

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 607 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu Glu Arg Leu Ile Arg Lys Phe Glu Ala Glu Asn Ile Ser Asn Tyr
1 5 10 15

Thr Ala Leu Leu Leu Ser Gln Asp Gly Lys Thr Leu Tyr Val Gly Ala
20 25 30

Arg Glu Ala Leu Phe Ala Leu Asn Ser Asn Leu Ser Phe Leu Pro Gly
35 40 45

Gly Glu Tyr Gln Glu Leu Leu Trp Ser Ala Asp Ala Asp Arg Lys Gln
50 55 60

Gln Cys Ser Phe Lys Gly Lys Asp Pro Lys Arg Asp Cys Gln Asn Tyr
65 70 75 80

Ile Lys Ile Leu Leu Pro Leu Asn Ser Ser His Leu Leu Thr Cys Gly
85 90 95

Thr Ala Ala Phe Ser Pro Leu Cys Ala Tyr Ile His Ile Ala Ser Phe
100 105 110

Thr Leu Ala Gln Asp Glu Ala Gly Asn Val Ile Leu Glu Asp Gly Lys
115 120 125

Gly His Cys Pro Phe Asp Pro Asn Phe Lys Ser Thr Ala Leu Val Val
130 135 140

Asp Gly Glu Leu Tyr Thr Gly Thr Val Ser Ser Phe Gln Gly Asn Asp
145 150 155 160

Pro Ala Ile Ser Arg Ser Gln Ser Ser Arg Pro Thr Lys Thr Glu Ser
165 170 175

Ser Leu Asn Trp Leu Gln Asp Pro Ala Phe Val Ala Ser Ala Thr Ser
180 185 190

Pro Glu Ser Leu Gly Ser Pro Ile Gly Asp Asp Asp Lys Ile Tyr Phe
195 200 205

Phe Phe Ser Glu Thr Gly Gln Glu Phe Glu Phe Phe Glu Asn Thr Ile
210 215 220

Val Ser Arg Val Ala Arg Val Cys Lys Gly Asp Glu Gly Gly Glu Arg
225 230 235 240

Val Leu Gln Gln Arg Trp Thr Ser Phe Leu Lys Ala Gln Leu Leu Cys
245 250 255

Ser Arg Pro Asp Asp Gly Phe Pro Phe Asn Val Leu Gln Asp Val Phe
260 265 270

Thr Leu Asn Pro Asn Pro Gln Asp Trp Arg Lys Thr Leu Ser Ile Gly
275 280 285

Val Phe Thr Ser Gln Trp His Arg Gly Thr Thr Glu Gly Ser Ala Ile
290 295 300

Cys Val Phe Thr Met Asn Asp Val Gln Lys Ala Phe Asp Gly Leu Tyr
305 310 315 320

Lys Lys Val Asn Arg Glu Thr Gln Gln Trp Tyr Thr Glu Thr His Gln
325 330 335

Val Pro Thr Pro Arg Pro Gly Ala Cys Ile Thr Asn Ser Ala Arg Glu
340 345 350

Arg Lys Ile Asn Ser Ser Leu Gln Leu Pro Asp Arg Val Leu Asn Phe
355 360 365

Leu Lys Asp His Phe Leu Met Asp Gly Gln Val Arg Ser Arg Leu Leu
370 375 380

Leu Leu Gln Pro Arg Ala Arg Tyr Gln Arg Val Ala Val His Arg Val
385 390 395 400

Pro Gly Leu His Ser Thr Tyr Asp Val Leu Phe Leu Gly Thr Gly Asp
405 410 415

Gly Arg Leu His Lys Ala Val Thr Leu Ser Ser Arg Val His Ile Ile
420 425 430

Glu Glu Leu Gln Ile Phe Pro Gln Gly Gln Pro Val Gln Asn Leu Leu
435 440 445

Leu Asp Ser His Gly Gly Leu Leu Tyr Ala Ser Ser His Ser Gly Val
450 455 460

Val Gln Val Pro Val Ala Asn Cys Ser Leu Tyr Pro Thr Cys Gly Asp
465 470 475 480

Cys Leu Leu Ala Arg Asp Pro Tyr Cys Ala Trp Thr Gly Ser Ala Cys
485 490 495

Arg Leu Ala Ser Leu Tyr Gln Pro Asp Leu Ala Ser Arg Pro Trp Thr
500 505 510

Gln Asp Ile Glu Gly Ala Ser Val Lys Glu Leu Cys Lys Asn Ser Ser
515 520 525

Tyr Lys Ala Arg Phe Leu Val Pro Gly Lys Pro Cys Lys Gln Val Gln
530 535 540

Ile Gln Pro Asn Thr Val Asn Thr Leu Ala Cys Pro Leu Leu Ser Asn
545 550 555 560

Leu Ala Thr Arg Leu Trp Val His Asn Gly Ala Pro Val Asn Ala Ser
565 570 575

Ala Ser Cys Arg Val Leu Pro Thr Gly Asp Leu Leu Leu Val Gly Ser
580 585 590

Gln Gln Gly Leu Gly Val Phe Gln Cys Trp Ser Ile Glu Glu Gly
595 600 605

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Lys Pro Ala Leu Asp Thr Gly Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 295 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asp Asp Lys Ile Tyr Phe Phe Phe Thr Glu Val Ser Val Glu Tyr Glu
1 5 10 15

Phe Val Gly Lys Leu Met Ile Pro Arg Ile Ala Arg Val Cys Lys Arg
20 25 30

Asp Gln Gly Gly Leu Arg Thr Leu Gln Lys Lys Trp Thr Ser Phe Leu
35 40 45

Lys Ala Arg Leu Ile Cys Thr Ile Pro Asp Lys Asn Leu Ile Phe Asn
50 55 60

Ile Ile Asn Asp Val Phe Thr Leu Lys Ser Pro Thr Leu Lys Glu Pro
65 70 75 80

Val Ile Tyr Gly Val Phe Thr Pro Gln Leu Asn Asn Val Gly Leu Ser
85 90 95

Ala Val Cys Ala Tyr Asn Leu Ser Ala Val Glu Glu Val Phe Ser Lys
100 105 110

Gly Lys Tyr Met Gln Ser Ala Thr Val Glu Gln Ser His Thr Lys Trp
115 120 125

Val Arg Tyr Asn Gly Glu Ile Pro Asn Pro Arg Pro Gly Ala Cys Ile
130 135 140

Asn Asn Glu Ala Arg Ala Leu Asn Tyr Val Thr Ser Leu Asn Leu Pro
145 150 155 160

Asp Lys Thr Leu Gln Phe Val Lys Asp His Pro Leu Met Asp Asp Ser
165 170 175

Val Thr Pro Val Gly Asp Arg Pro Arg Leu Val Lys Arg Asp Val Lys
180 185 190

Tyr Thr Gln Ile Val Val Asp Arg Val Arg Ala Leu Asn Gly Thr Ile
195 200 205

Tyr Asp Val Met Phe Ile Ser Thr Asp Gln Gly Ala Leu His Lys Ala
210 215 220

Ile Ser Tyr Glu Asn Gly Met His Ile Ile Glu Glu Thr Gln Leu Phe
225 230 235 240

Pro Lys Phe Glu Pro Val Gln Thr Leu Leu Leu Ser Ser Lys Lys Ser
245 250 255

Arg Arg Tyr Leu Tyr Ala Gly Ser Asn Ser Gly Val Val Gln Ser Pro
260 265 270

Val Ala Phe Cys Asp Thr Tyr Thr Thr Cys Phe Asp Cys Val Leu Ala
275 280 285

Arg Asp Pro Tyr Cys Ala Trp
290 295

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 425 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Glu Leu Tyr Ser Gly Thr Ser Tyr Asn Phe Leu Gly Ser Glu Pro
1 5 10 15

Ile Ile Ser Arg Asn Ser Ser His Ser Pro Leu Arg Thr Glu Tyr Ala
20 25 30

Ile Pro Trp Leu Asn Glu Pro Ser Phe Val Phe Ala Asp Val Ile Arg
35 40 45

Lys Ser Pro Asp Ser Pro Asp Gly Glu Asp Asp Arg Val Tyr Phe Phe
50 55 60

Phe Thr Glu Val Ser Val Glu Tyr Glu Phe Val Phe Arg Val Leu Ile
65 70 75 80

Pro Arg Ile Ala Arg Val Cys Lys Gly Asp Gln Gly Gly Leu Arg Thr
85 90 95

Leu Gln Lys Lys Trp Thr Ser Phe Leu Lys Ala Arg Leu Ile Cys Ser
100 105 110

Arg Pro Asp Ser Gly Leu Val Phe Asn Val Leu Arg Asp Val Phe Val
115 120 125

Leu Arg Ser Pro Gly Leu Lys Val Pro Val Phe Tyr Ala Leu Phe Thr
130 135 140

Pro Gln Leu Asn Asn Val Gly Leu Ser Ala Val Cys Ala Tyr Asn Leu
145 150 155 160

Ser Thr Ala Glu Glu Val Phe Ser His Gly Lys Tyr Met Gln Ser Thr
165 170 175

Thr Val Glu Gln Ser His Thr Lys Trp Val Arg Tyr Asn Gly Pro Val
180 185 190

Pro Lys Pro Arg Pro Gly Ala Cys Ile Asp Ser Glu Ala Arg Ala Ala
195 200 205

Asn Tyr Thr Ser Ser Leu Asn Leu Pro Asp Lys Thr Leu Gln Phe Val
210 215 220

Lys Asp His Pro Leu Met Asp Asp Ser Val Thr Pro Ile Asp Asn Arg
225 230 235 240

Pro Arg Leu Ile Lys Lys Asp Val Asn Tyr Thr Gln Ile Val Val Asp
245 250 255

Arg Thr Gln Ala Leu Asp Gly Thr Val Tyr Asp Val Met Phe Val Ser
260 265 270

Thr Asp Arg Gly Ala Leu His Lys Ala Ile Ser Leu Glu His Ala Val
275 280 285

His Ile Ile Glu Glu Thr Gln Leu Phe Gln Asp Phe Glu Pro Val Gln
290 295 300

Thr Leu Leu Leu Ser Ser Lys Lys Gly Asn Arg Phe Val Tyr Ala Gly
305 310 315 320

Ser Asn Ser Gly Val Val Gln Ala Pro Leu Ala Phe Cys Gly Lys His
325 330 335

Gly Thr Cys Glu Asp Cys Val Leu Ala Arg Asp Pro Tyr Cys Ala Trp
340 345 350

Ser Pro Pro Thr Ala Thr Cys Val Ala Leu His Gln Thr Glu Ser Pro
355 360 365

Ser Arg Gly Leu Ile Gln Glu Met Ser Gly Asp Ala Ser Val Cys Pro
370 375 380

Asp Lys Ser Lys Gly Ser Tyr Arg Gln His Phe Phe Lys His Gly Gly
385 390 395 400

Thr Ala Glu Leu Lys Cys Ser Gln Lys Ser Asn Leu Ala Arg Val Phe
405 410 415

Trp Lys Phe Gln Asn Gly Val Leu Lys
420 425

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4391 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 434..3017

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
CATCTCCGCG CGTCTCCCGG CCTCCCCGAC TCAAGCTCCT GGCGCCGTTC GCCTCTGTCG      60

TCGCTGCCAC CAACGCCGCC GCCATTCTTG GCGCGGAGCT GGCCTGGCCA GCCCCCGCCG     120

AGAGCCTCCG CGGGTCGGAC GCGCGCTCCA GACCCTGGTA ACACCACAGG AACACAGCCA     180

AGTCCTGAAT ACCGACATGT CTGACAGTGC TGATCCCTAG GTCAGACGGG AACACCGGCA     240

GCCTTGGCAT GACGTCGTGA AGGTGGCCAT TGCTAACCTG ACATGTGGGG ACTCAGGAAC     300

CCCACCCCTT ATGGGCTCCA GTCTGTGCTG CTGGCCCCAG CTCTGGGGCT CTAAGAGGTC     360

CTTGCTGCTA CCCCACAGCA GCCTGCTGCC ATCCATGTGT GCCCGTTGCT GAAGGCCTCG     420

GTGGCCCCTG CCC ATG AGG ATG TGT GCC CCC GTT AGG GGG CTG TTC TTG          469
                Met Arg Met Cys Ala Pro Val Arg Gly Leu Phe Leu
                  1               5                  10

GCC CTG GTG GTA GTG TTG AGA ACC GCG GTG GCA TTT GCA CCT GTG CCT        517
Ala Leu Val Val Val Leu Arg Thr Ala Val Ala Phe Ala Pro Val Pro
          15                  20                  25

CGG CTC ACC TGG GAA CAT GGA GAG GTA GGT CTG GTG CAG TTT CAC AAG        565
Arg Leu Thr Trp Glu His Gly Glu Val Gly Leu Val Gln Phe His Lys
     30                  35                  40

CCA GGC ATC TTT AAC TAC TCG GCC TTG CTG ATG AGT GAG GAC AAA GAC        613
Pro Gly Ile Phe Asn Tyr Ser Ala Leu Leu Met Ser Glu Asp Lys Asp
 45                  50                  55                  60

ACT CTG TAT GTA GGC GCC CGG GAA GCA GTC TTT GCA GTG AAT GCG CTG        661
Thr Leu Tyr Val Gly Ala Arg Glu Ala Val Phe Ala Val Asn Ala Leu
                  65                  70                  75

AAC ATC TCT GAG AAG CAA CAT GAG GTA TAT TGG AAG GTC TCT GAA GAC        709
Asn Ile Ser Glu Lys Gln His Glu Val Tyr Trp Lys Val Ser Glu Asp
             80                  85                  90

AAA AAA TCC AAG TGT GCA GAG AAG GGG AAA TCA AAG CAG ACG GAA TGC        757
Lys Lys Ser Lys Cys Ala Glu Lys Gly Lys Ser Lys Gln Thr Glu Cys
          95                  100                 105

CTA AAC TAC ATT CGA GTA CTA CAG CCA CTA AGC AGC ACT TCC CTC TAT        805
Leu Asn Tyr Ile Arg Val Leu Gln Pro Leu Ser Ser Thr Ser Leu Tyr
     110                 115                 120

GTG TGT GGG ACC AAT GCG TTC CAG CCC ACC TGT GAC CAC CTG AAC TTG        853
Val Cys Gly Thr Asn Ala Phe Gln Pro Thr Cys Asp His Leu Asn Leu
125                 130                 135                 140

ACA TCC TTC AAG TTT CTG GGG AAA AGT GAA GAT GGC AAA GGA AGA TGC        901
Thr Ser Phe Lys Phe Leu Gly Lys Ser Glu Asp Gly Lys Gly Arg Cys
                145                 150                 155
```

```
CCC TTC GAC CCC GCC CAC AGC TAC ACA TCA GTC ATG GTT GGG GGC GAG      949
Pro Phe Asp Pro Ala His Ser Tyr Thr Ser Val Met Val Gly Gly Glu
            160                 165                 170

CTC TAC TCT GGG ACG TCC TAT AAT TTC TTG GGC AGT GAA CCC ATC ATC      997
Leu Tyr Ser Gly Thr Ser Tyr Asn Phe Leu Gly Ser Glu Pro Ile Ile
        175                 180                 185

TCT CGA AAC TCT TCC CAC AGT CCC TTG AGG ACG GAG TAT GCC ATC CCG     1045
Ser Arg Asn Ser Ser His Ser Pro Leu Arg Thr Glu Tyr Ala Ile Pro
    190                 195                 200

TGG CTG AAC GAG CCT AGC TTC GTC TTT GCT GAC GTG ATC CAG AAA AGC     1093
Trp Leu Asn Glu Pro Ser Phe Val Phe Ala Asp Val Ile Gln Lys Ser
205                 210                 215                 220

CCA GAT GGT CCG GAG GGT GAA GAT GAC AAG GTC TAC TTC TTT TTT ACG     1141
Pro Asp Gly Pro Glu Gly Glu Asp Asp Lys Val Tyr Phe Phe Phe Thr
                225                 230                 235

GAG GTA TCC GTG GAG TAC GAA TTC GTC TTC AAG TTG ATG ATC CCG CGA     1189
Glu Val Ser Val Glu Tyr Glu Phe Val Phe Lys Leu Met Ile Pro Arg
            240                 245                 250

GTT GCC AGG GTG TGC AAG GGC GAC CAG GGC GGC CTG CGG ACT TTG CAA     1237
Val Ala Arg Val Cys Lys Gly Asp Gln Gly Gly Leu Arg Thr Leu Gln
        255                 260                 265

AAA AAG TGG ACC TCC TTC CTA AAG GCC AGG CTG ATC TGC TCC AAG CCA     1285
Lys Lys Trp Thr Ser Phe Leu Lys Ala Arg Leu Ile Cys Ser Lys Pro
    270                 275                 280

GAC AGT GGC CTG GTC TTC AAC ATA CTT CAG GAT GTG TTT GTG CTG AGG     1333
Asp Ser Gly Leu Val Phe Asn Ile Leu Gln Asp Val Phe Val Leu Arg
285                 290                 295                 300

GCC CCG GGC CTC AAG GAG CCT GTG TTC TAT GCG GTC TTC ACC CCA CAG     1381
Ala Pro Gly Leu Lys Glu Pro Val Phe Tyr Ala Val Phe Thr Pro Gln
                305                 310                 315

CTG AAC AAT GTG GGT CTG TCA GCG GTG TGC GCC TAC ACA CTG GCC ACG     1429
Leu Asn Asn Val Gly Leu Ser Ala Val Cys Ala Tyr Thr Leu Ala Thr
            320                 325                 330

GTG GAG GCA GTC TTC TCC CGT GGA AAG TAC ATG CAG AGT GCC ACA GTG     1477
Val Glu Ala Val Phe Ser Arg Gly Lys Tyr Met Gln Ser Ala Thr Val
        335                 340                 345

GAG CAG TCT CAC ACC AAG TGG GTG CGC TAC AAT GGC CCA GTG CCC ACT     1525
Glu Gln Ser His Thr Lys Trp Val Arg Tyr Asn Gly Pro Val Pro Thr
    350                 355                 360

CCC CGA CCT GGA GCG TGT ATC GAC AGT GAG GCC CGG GCA GCC AAC TAC     1573
Pro Arg Pro Gly Ala Cys Ile Asp Ser Glu Ala Arg Ala Ala Asn Tyr
365                 370                 375                 380
```

```
ACC AGC TCC TTG AAT CTC CCA GAC AAA ACA CTG CAG TTT GTA AAA GAC      1621
Thr Ser Ser Leu Asn Leu Pro Asp Lys Thr Leu Gln Phe Val Lys Asp
                385                 390                 395

CAC CCT TTG ATG GAT GAC TCA GTG ACC CCG ATA GAC AAC AGA CCC AAG      1669
His Pro Leu Met Asp Asp Ser Val Thr Pro Ile Asp Asn Arg Pro Lys
            400                 405                 410

CTG ATC AAA AAA GAT GTA AAC TAC ACC CAG ATA GTG GTA GAC AGG ACC      1717
Leu Ile Lys Lys Asp Val Asn Tyr Thr Gln Ile Val Val Asp Arg Thr
        415                 420                 425

CAG GCC CTG GAT GGG ACT TTC TAC GAC GTC ATG TTC ATC AGC ACA GAC      1765
Gln Ala Leu Asp Gly Thr Phe Tyr Asp Val Met Phe Ile Ser Thr Asp
    430                 435                 440

CGG GGA GCT CTG CAT AAA GCA GTC ATC CTC ACA AAA GAG GTG CAT GTC      1813
Arg Gly Ala Leu His Lys Ala Val Ile Leu Thr Lys Glu Val His Val
445                 450                 455                 460

ATC GAG GAG ACC CAA CTC TTC CGG GAC TCT GAA CCG GTC CTA ACT CTG      1861
Ile Glu Glu Thr Gln Leu Phe Arg Asp Ser Glu Pro Val Leu Thr Leu
                465                 470                 475

CTG CTA TCG TCA AAG AAG GGG AGG AAG TTT GTC TAT GCA GGC TCC AAC      1909
Leu Leu Ser Ser Lys Lys Gly Arg Lys Phe Val Tyr Ala Gly Ser Asn
            480                 485                 490

TCT GGA GTG GTC CAA GCG CCC CTG GCA TTC TGC GAA AAG CAC GGT AGC      1957
Ser Gly Val Val Gln Ala Pro Leu Ala Phe Cys Glu Lys His Gly Ser
        495                 500                 505

TGT GAA GAC TGT GTG TTA GCA CGG GAC CCC TAC TGT GCC TGG AGC CCA      2005
Cys Glu Asp Cys Val Leu Ala Arg Asp Pro Tyr Cys Ala Trp Ser Pro
    510                 515                 520

GCC ATC AAG GCC TGT GTT ACC CTG CAC CAG GAA GAG GCC TCC AGC AGG      2053
Ala Ile Lys Ala Cys Val Thr Leu His Gln Glu Glu Ala Ser Ser Arg
525                 530                 535                 540

GGC TGG ATT CAG GAC ATG AGC GGT GAC ACA TCC TCA TGC CTG GAT AAG      2101
Gly Trp Ile Gln Asp Met Ser Gly Asp Thr Ser Ser Cys Leu Asp Lys
                545                 550                 555

AGT AAA GAA AGT TTC AAC CAG CAT TTT TTC AAG CAC GGC GGC ACA GCG      2149
Ser Lys Glu Ser Phe Asn Gln His Phe Phe Lys His Gly Gly Thr Ala
            560                 565                 570

GAA CTC AAA TGT TTC CAA AAG TCC AAC CTA GCC CGG GTG GTA TGG AAG      2197
Glu Leu Lys Cys Phe Gln Lys Ser Asn Leu Ala Arg Val Val Trp Lys
        575                 580                 585

TTC CAG AAT GGC GAG TTG AAG GCC GCA AGT CCC AAG TAC GGC TTT GTG      2245
Phe Gln Asn Gly Glu Leu Lys Ala Ala Ser Pro Lys Tyr Gly Phe Val
    590                 595                 600
```

```
GGC AGG AAG CAC CTG CTC ATC TTC AAC CTG TCG GAC GGA GAC AGC GGC      2293
Gly Arg Lys His Leu Leu Ile Phe Asn Leu Ser Asp Gly Asp Ser Gly
605                 610                 615                 620

GTG TAC CAG TGC CTG TCA GAG GAA AGG GTG AGG AAT AAA ACG GTC TCC      2341
Val Tyr Gln Cys Leu Ser Glu Glu Arg Val Arg Asn Lys Thr Val Ser
                625                 630                 635

CAG CTG CTG GCC AAG CAC GTT CTG GAA GTG AAG ATG GTA CCT CGG ACC      2389
Gln Leu Leu Ala Lys His Val Leu Glu Val Lys Met Val Pro Arg Thr
            640                 645                 650

CCC CCC TCA CCT ACC TCA GAG GAT GCT CAG ACA GAA GGT AGT AAG ATC      2437
Pro Pro Ser Pro Thr Ser Glu Asp Ala Gln Thr Glu Gly Ser Lys Ile
        655                 660                 665

ACA TCC AAA ATG CCG GTT GCA TCT ACC CAG GGG TCC TCT CCC CCT ACC      2485
Thr Ser Lys Met Pro Val Ala Ser Thr Gln Gly Ser Ser Pro Pro Thr
    670                 675                 680

CCG GCT CTG TGG GCA ACC TCC CCC AGA GCC GCC ACC CTA CCT CCC AAG      2533
Pro Ala Leu Trp Ala Thr Ser Pro Arg Ala Ala Thr Leu Pro Pro Lys
685                 690                 695                 700

TCC TCC TCC GGC ACA TCC TGT GAA CCA AAG ATG GTC ATC AAC ACG GTC      2581
Ser Ser Ser Gly Thr Ser Cys Glu Pro Lys Met Val Ile Asn Thr Val
                705                 710                 715

CCC CAG CTC CAC TCA GAG AAG ACG GTG TAT CTC AAG TCC AGT GAC AAC      2629
Pro Gln Leu His Ser Glu Lys Thr Val Tyr Leu Lys Ser Ser Asp Asn
            720                 725                 730

CGC CTG CTC ATG TCT CTC CTC CTC TTC ATC TTT GTC CTC TTC CTC TGC      2677
Arg Leu Leu Met Ser Leu Leu Leu Phe Ile Phe Val Leu Phe Leu Cys
        735                 740                 745

CTC TTT TCC TAC AAC TGC TAC AAG GGC TAC CTG CCC GGA CAG TGC TTA      2725
Leu Phe Ser Tyr Asn Cys Tyr Lys Gly Tyr Leu Pro Gly Gln Cys Leu
    750                 755                 760

AAA TTC CGC TCA GCC CTG CTG CTT GGA AAG AAA ACA CCC AAG TCA GAC      2773
Lys Phe Arg Ser Ala Leu Leu Leu Gly Lys Lys Thr Pro Lys Ser Asp
765                 770                 775                 780

TTC TCT GAC CTG GAG CAG AGT GTG AAG GAG ACA CTG GTC GAG CCT GGG      2821
Phe Ser Asp Leu Glu Gln Ser Val Lys Glu Thr Leu Val Glu Pro Gly
                785                 790                 795

AGC TTC TCC CAG CAG AAC GGC GAC CAC CCC AAG CCA GCC CTG GAT ACG      2869
Ser Phe Ser Gln Gln Asn Gly Asp His Pro Lys Pro Ala Leu Asp Thr
            800                 805                 810

GGC TAT GAA ACG GAG CAG GAC ACC ATC ACC AGC AAA GTC CCC ACG GAT      2917
Gly Tyr Glu Thr Glu Gln Asp Thr Ile Thr Ser Lys Val Pro Thr Asp
        815                 820                 825
```

```
CGT GAG GAC TCG CAA CGG ATC GAT GAA CTC TCT GCC CGG GAC AAA CCG      2965
Arg Glu Asp Ser Gln Arg Ile Asp Glu Leu Ser Ala Arg Asp Lys Pro
    830                 835                 840

TTT GAT GTC AAG TGT GAA CTG AAG TTT GCA GAT TCG GAT GCT GAC GGG      3013
Phe Asp Val Lys Cys Glu Leu Lys Phe Ala Asp Ser Asp Ala Asp Gly
845                 850                 855                 860

GAC T GAGGCCAGCG TGTCCCAGCC CATGCCCCTC TGTCTTCGTG GAGAGTGTTG        3067
Asp   *

TGTTGAGCCC ATTCAGTAGC CGAGTCTTGT CACTCTGTGC CAGCCTCAGT CCTGTGTCCC   3127

TTTTTCTCTT GGGTTGAGCC TGTGGCTCAT CCCCTTTGTC CTTTTGGGAA GCAAGTATCT   3187

ATTCCAGTCT CAAGTCCTGC AGTTGCTGGA GCGCTTACGC ACCTGAGCCC TTTGTGTCCT   3247

GGGGGAGAGA TGGCCACCTC CGTGGGCTGC GAAGAGCCAC CCCTTCCTCT TCCGATTCTC   3307

CTAGCAGCCA CTCAGAGATA ATTTAATTCC AGATTGGAAA CGCCCTTTTA GTTTATCAGA   3367

TTGGTAACTT ACATCCTGCT GCCCAGATGG CACGGACAGT TTTCTTTCAC TTAATTATTA   3427

TTTTTTTTTT AAGGATTTTC GCTCCTATTG TGTTGATGTC TTAGGTCATT TTCTTTTTTT   3487

CTTTCTCTTT TTTTATTACC AGAGGAGATG TTTTAATATT CATGAGAAGA GGAACATTTT   3547

CTAGATTTTT TTGTTGTTAT ATATTGAGAT ATAAAATATG GCTATGTTGC TTAAGATTCT   3607

CAGGGATAGA CTTATTTTTG TTAACTTCAT TCTTTCCTGC TGTTAGGAAC ATAGGCCTAA   3667

AATTGTCTCT TGAGTTTGCT CACCCTTTTG TTTTGGTAGG GTTTTTTTGT TGTTGCTGTT   3727

ATTGTTTCTA GTTTTTAATC TTATTCATTT TGAAGGATTT TTCTTTCTGA ACTTTTTAAA   3787

TTTTTATATT TTCCTGCCAT ACATCTACAA AGTGGGTTTT GAGTGAGGGC AGGTGGCCCA   3847

GTGGCTTTGG GTGGCGACTG AGCTGGTCCC ACGAGGGGAG GAGGGTTTAT ATACCCCATG   3907

ACCCTGCGGC TTCTTGGCGC CTCCTGCCCA TGAGGATCAC ATCCTGTCTC TCCTTGCTTC   3967

CATCTCTCAT CACTGCCCTT GGACTTCCGC CTTGACTGTC CATGAAAGAC AGAAATGGGT   4027

TGGGTAGTTG GGCTCCCAAC CTCGGATGGT GACCGCAAAT CCCGGTGGGC GGCCGGCCGT   4087

CTGCAGCCCG ATTCCTGCCA GTGTTTTCAG GATGTAACGG GGGTACGATT TGGCATTTGT   4147

TTTTCGCTCC CGGGTGGAAA TCATTCCATG TGGGGGACGA ATTTTGGATT CCCCCCCACC   4207

CCCACCCCGG CGGGTAGAAA TTTTTTTGCA TGACAGATTT ACCATTCGGT GTAAAATTTG   4267

GTTTATAAGA TTTATTTGTT TTTATTTTTT TATGGACTGT AAATTTGAAA GTACCCAAAT   4327

AAACCGAGTT TTAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA   4387

AAAA                                                                4391
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 861 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Met Arg Met Cys Ala Pro Val Arg Gly Leu Phe Leu Ala Leu Val Val
 1               5                  10                  15

Val Leu Arg Thr Ala Val Ala Phe Ala Pro Val Pro Arg Leu Thr Trp
            20                  25                  30

Glu His Gly Glu Val Gly Leu Val Gln Phe His Lys Pro Gly Ile Phe
        35                  40                  45

Asn Tyr Ser Ala Leu Leu Met Ser Glu Asp Lys Asp Thr Leu Tyr Val
    50                  55                  60

Gly Ala Arg Glu Ala Val Phe Ala Val Asn Ala Leu Asn Ile Ser Glu
65                  70                  75                  80

Lys Gln His Glu Val Tyr Trp Lys Val Ser Glu Asp Lys Lys Ser Lys
                85                  90                  95

Cys Ala Glu Lys Gly Lys Ser Lys Gln Thr Glu Cys Leu Asn Tyr Ile
            100                 105                 110

Arg Val Leu Gln Pro Leu Ser Ser Thr Ser Leu Tyr Val Cys Gly Thr
        115                 120                 125

Asn Ala Phe Gln Pro Thr Cys Asp His Leu Asn Leu Thr Ser Phe Lys
    130                 135                 140

Phe Leu Gly Lys Ser Glu Asp Gly Lys Gly Arg Cys Pro Phe Asp Pro
145                 150                 155                 160

Ala His Ser Tyr Thr Ser Val Met Val Gly Gly Glu Leu Tyr Ser Gly
                165                 170                 175

Thr Ser Tyr Asn Phe Leu Gly Ser Glu Pro Ile Ile Ser Arg Asn Ser
            180                 185                 190

Ser His Ser Pro Leu Arg Thr Glu Tyr Ala Ile Pro Trp Leu Asn Glu
        195                 200                 205

Pro Ser Phe Val Phe Ala Asp Val Ile Gln Lys Ser Pro Asp Gly Pro
    210                 215                 220

Glu Gly Glu Asp Asp Lys Val Tyr Phe Phe Phe Thr Glu Val Ser Val
225                 230                 235                 240
```

```
Glu Tyr Glu Phe Val Phe Lys Leu Met Ile Pro Arg Val Ala Arg Val
                245                 250                 255

Cys Lys Gly Asp Gln Gly Gly Leu Arg Thr Leu Gln Lys Lys Trp Thr
            260                 265                 270

Ser Phe Leu Lys Ala Arg Leu Ile Cys Ser Lys Pro Asp Ser Gly Leu
        275                 280                 285

Val Phe Asn Ile Leu Gln Asp Val Phe Val Leu Arg Ala Pro Gly Leu
    290                 295                 300

Lys Glu Pro Val Phe Tyr Ala Val Phe Thr Pro Gln Leu Asn Asn Val
305                 310                 315                 320

Gly Leu Ser Ala Val Cys Ala Tyr Thr Leu Ala Thr Val Glu Ala Val
                325                 330                 335

Phe Ser Arg Gly Lys Tyr Met Gln Ser Ala Thr Val Glu Gln Ser His
            340                 345                 350

Thr Lys Trp Val Arg Tyr Asn Gly Pro Val Pro Thr Pro Arg Pro Gly
        355                 360                 365

Ala Cys Ile Asp Ser Glu Ala Arg Ala Ala Asn Tyr Thr Ser Ser Leu
    370                 375                 380

Asn Leu Pro Asp Lys Thr Leu Gln Phe Val Lys Asp His Pro Leu Met
385                 390                 395                 400

Asp Asp Ser Val Thr Pro Ile Asp Asn Arg Pro Lys Leu Ile Lys Lys
                405                 410                 415

Asp Val Asn Tyr Thr Gln Ile Val Val Asp Arg Thr Gln Ala Leu Asp
            420                 425                 430

Gly Thr Phe Tyr Asp Val Met Phe Ile Ser Thr Asp Arg Gly Ala Leu
        435                 440                 445

His Lys Ala Val Ile Leu Thr Lys Glu Val His Val Ile Glu Glu Thr
    450                 455                 460

Gln Leu Phe Arg Asp Ser Glu Pro Val Leu Thr Leu Leu Leu Ser Ser
465                 470                 475                 480

Lys Lys Gly Arg Lys Phe Val Tyr Ala Gly Ser Asn Ser Gly Val Val
                485                 490                 495

Gln Ala Pro Leu Ala Phe Cys Glu Lys His Gly Ser Cys Glu Asp Cys
            500                 505                 510

Val Leu Ala Arg Asp Pro Tyr Cys Ala Trp Ser Pro Ala Ile Lys Ala
        515                 520                 525

Cys Val Thr Leu His Gln Glu Glu Ala Ser Ser Arg Gly Trp Ile Gln
    530                 535                 540
```

```
Asp Met Ser Gly Asp Thr Ser Ser Cys Leu Asp Lys Ser Lys Glu Ser
545                 550                 555                 560

Phe Asn Gln His Phe Phe Lys His Gly Gly Thr Ala Glu Leu Lys Cys
                565                 570                 575

Phe Gln Lys Ser Asn Leu Ala Arg Val Val Trp Lys Phe Gln Asn Gly
            580                 585                 590

Glu Leu Lys Ala Ala Ser Pro Lys Tyr Gly Phe Val Gly Arg Lys His
        595                 600                 605

Leu Leu Ile Phe Asn Leu Ser Asp Gly Asp Ser Gly Val Tyr Gln Cys
    610                 615                 620

Leu Ser Glu Glu Arg Val Arg Asn Lys Thr Val Ser Gln Leu Leu Ala
625                 630                 635                 640

Lys His Val Leu Glu Val Lys Met Val Pro Arg Thr Pro Pro Ser Pro
                645                 650                 655

Thr Ser Glu Asp Ala Gln Thr Glu Gly Ser Lys Ile Thr Ser Lys Met
            660                 665                 670

Pro Val Ala Ser Thr Gln Gly Ser Ser Pro Pro Thr Pro Ala Leu Trp
        675                 680                 685

Ala Thr Ser Pro Arg Ala Ala Thr Leu Pro Pro Lys Ser Ser Ser Gly
    690                 695                 700

Thr Ser Cys Glu Pro Lys Met Val Ile Asn Thr Val Pro Gln Leu His
705                 710                 715                 720

Ser Glu Lys Thr Val Tyr Leu Lys Ser Ser Asp Asn Arg Leu Leu Met
                725                 730                 735

Ser Leu Leu Leu Phe Ile Phe Val Leu Phe Leu Cys Leu Phe Ser Tyr
            740                 745                 750

Asn Cys Tyr Lys Gly Tyr Leu Pro Gly Gln Cys Leu Lys Phe Arg Ser
        755                 760                 765

Ala Leu Leu Leu Gly Lys Lys Thr Pro Lys Ser Asp Phe Ser Asp Leu
    770                 775                 780

Glu Gln Ser Val Lys Glu Thr Leu Val Glu Pro Gly Ser Phe Ser Gln
785                 790                 795                 800

Gln Asn Gly Asp His Pro Lys Pro Ala Leu Asp Thr Gly Tyr Glu Thr
                805                 810                 815

Glu Gln Asp Thr Ile Thr Ser Lys Val Pro Thr Asp Arg Glu Asp Ser
            820                 825                 830

Gln Arg Ile Asp Glu Leu Ser Ala Arg Asp Lys Pro Phe Asp Val Lys
        835                 840                 845
```

Cys Glu Leu Lys Phe Ala Asp Ser Asp Ala Asp Gly Asp
850 855 860

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Arg Met Cys Ala Pro Val Arg Gly Leu Phe Leu Ala Leu Val Val
1 5 10 15

Val Leu Arg Thr Ala Val Ala Phe Ala Pro Val Pro Arg Leu Thr Trp
20 25 30

Glu His Gly Glu Val Gly Leu Val Gln
35 40

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Arg Met Cys Thr Pro Ile Arg Gly Leu Leu Met Ala Leu Ala Val
1 5 10 15

Met Phe Gly Thr Ala Met Ala Phe Ala Pro Ile Pro Arg Ile Thr Trp
20 25 30

Glu His Arg Glu Val His Leu Val Gln
35 40

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 512 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Phe His Lys Pro Gly Ile Phe Asn Tyr Ser Ala Leu Leu Met Ser Glu
1               5                  10                  15

Asp Lys Asp Thr Leu Tyr Val Gly Ala Arg Glu Ala Val Phe Ala Val
            20                  25                  30

Asn Ala Leu Asn Ile Ser Glu Lys Gln His Glu Val Tyr Trp Lys Val
        35                  40                  45

Ser Glu Asp Lys Lys Ser Lys Cys Ala Glu Lys Gly Lys Ser Lys Gln
    50                  55                  60

Thr Glu Cys Leu Asn Tyr Ile Arg Val Leu Gln Pro Leu Ser Ser Thr
65                  70                  75                  80

Ser Leu Tyr Val Cys Gly Thr Asn Ala Phe Gln Pro Thr Cys Asp His
                85                  90                  95

Leu Asn Leu Thr Ser Phe Lys Phe Leu Gly Lys Ser Glu Asp Gly Lys
            100                 105                 110

Gly Arg Cys Pro Phe Asp Pro Ala His Ser Tyr Thr Ser Val Met Val
        115                 120                 125

Gly Gly Glu Leu Tyr Ser Gly Thr Ser Tyr Asn Phe Leu Gly Ser Glu
    130                 135                 140

Pro Ile Ile Ser Arg Asn Ser Ser His Ser Pro Leu Arg Thr Glu Tyr
145                 150                 155                 160

Ala Ile Pro Trp Leu Asn Glu Pro Ser Phe Val Phe Ala Asp Val Ile
                165                 170                 175

Gln Lys Ser Pro Asp Gly Pro Glu Gly Glu Asp Asp Lys Val Tyr Phe
            180                 185                 190

Phe Phe Thr Glu Val Ser Val Glu Tyr Glu Phe Val Phe Lys Leu Met
        195                 200                 205

Ile Pro Arg Val Ala Arg Val Cys Lys Gly Asp Gln Gly Gly Leu Arg
    210                 215                 220

Thr Leu Gln Lys Lys Trp Thr Ser Phe Leu Lys Ala Arg Leu Ile Cys
225                 230                 235                 240

Ser Lys Pro Asp Ser Gly Leu Val Phe Asn Ile Leu Gln Asp Val Phe
                245                 250                 255
```

Val Leu Arg Ala Pro Gly Leu Lys Glu Pro Val Phe Tyr Ala Val Phe
260 265 270

Thr Pro Gln Leu Asn Asn Val Gly Leu Ser Ala Val Cys Ala Tyr Thr
275 280 285

Leu Ala Thr Val Glu Ala Val Phe Ser Arg Gly Lys Tyr Met Gln Ser
290 295 300

Ala Thr Val Glu Gln Ser His Thr Lys Trp Val Arg Tyr Asn Gly Pro
305 310 315 320

Val Pro Thr Pro Arg Pro Gly Ala Cys Ile Asp Ser Glu Ala Arg Ala
325 330 335

Ala Asn Tyr Thr Ser Ser Leu Asn Leu Pro Asp Lys Thr Leu Gln Phe
340 345 350

Val Lys Asp His Pro Leu Met Asp Asp Ser Val Thr Pro Ile Asp Asn
355 360 365

Arg Pro Lys Leu Ile Lys Lys Asp Val Asn Tyr Thr Gln Ile Val Val
370 375 380

Asp Arg Thr Gln Ala Leu Asp Gly Thr Phe Tyr Asp Val Met Phe Ile
385 390 395 400

Ser Thr Asp Arg Gly Ala Leu His Lys Ala Val Ile Leu Thr Lys Glu
405 410 415

Val His Val Ile Glu Glu Thr Gln Leu Phe Arg Asp Ser Glu Pro Val
420 425 430

Leu Thr Leu Leu Leu Ser Ser Lys Lys Gly Arg Lys Phe Val Tyr Ala
435 440 445

Gly Ser Asn Ser Gly Val Val Gln Ala Pro Leu Ala Phe Cys Glu Lys
450 455 460

His Gly Ser Cys Glu Asp Cys Val Leu Ala Arg Asp Pro Tyr Cys Ala
465 470 475 480

Trp Ser Pro Ala Ile Lys Ala Cys Val Thr Leu His Gln Glu Glu Ala
485 490 495

Ser Ser Arg Gly Trp Ile Gln Asp Met Ser Gly Asp Thr Ser Ser Cys
500 505 510

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 512 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Phe His Glu Pro Asp Ile Tyr Asn Tyr Ser Ala Leu Leu Leu Ser Glu
1 5 10 15

Asp Lys Asp Thr Leu Tyr Ile Gly Ala Arg Glu Ala Val Phe Ala Val
20 25 30

Asn Ala Leu Asn Ile Ser Glu Lys Gln His Glu Val Tyr Trp Lys Val
35 40 45

Ser Glu Asp Lys Lys Ala Lys Cys Ala Glu Lys Gly Lys Ser Lys Gln
50 55 60

Thr Glu Cys Leu Asn Tyr Ile Arg Val Leu Gln Pro Leu Ser Ala Thr
65 70 75 80

Ser Leu Tyr Val Cys Gly Thr Asn Ala Phe Gln Pro Ala Cys Asp His
85 90 95

Leu Asn Leu Thr Ser Phe Lys Phe Leu Gly Lys Asn Glu Asp Gly Lys
100 105 110

Gly Arg Cys Pro Phe Asp Pro Ala His Ser Tyr Thr Ser Val Met Val
115 120 125

Asp Gly Glu Leu Tyr Ser Gly Thr Ser Tyr Asn Phe Leu Gly Ser Glu
130 135 140

Pro Ile Ile Ser Arg Asn Ser Ser His Ser Pro Leu Arg Thr Glu Tyr
145 150 155 160

Ala Ile Pro Trp Leu Asn Glu Pro Ser Phe Val Phe Ala Asp Val Ile
165 170 175

Arg Lys Ser Pro Asp Ser Pro Asp Gly Glu Asp Asp Arg Val Tyr Phe
180 185 190

Phe Phe Thr Glu Val Ser Val Glu Tyr Glu Phe Val Phe Arg Val Leu
195 200 205

Ile Pro Arg Ile Ala Arg Val Cys Lys Gly Asp Gln Gly Gly Leu Arg
210 215 220

Thr Leu Gln Lys Lys Trp Thr Ser Phe Leu Lys Ala Arg Leu Ile Cys
225 230 235 240

Ser Arg Pro Asp Ser Gly Leu Val Phe Asn Val Leu Arg Asp Val Phe
245 250 255

Val Leu Arg Ser Pro Gly Leu Lys Val Pro Val Phe Tyr Ala Leu Phe
260 265 270

Thr Pro Gln Leu Asn Asn Val Gly Leu Ser Ala Val Cys Ala Tyr Asn
275 280 285

Leu Ser Thr Ala Glu Glu Val Phe Ser His Gly Lys Tyr Met Gln Ser
290 295 300

Thr Thr Val Glu Gln Ser His Thr Lys Trp Val Arg Tyr Asn Gly Pro
305 310 315 320

Val Pro Lys Pro Arg Pro Gly Ala Cys Ile Asp Ser Glu Ala Arg Ala
325 330 335

Ala Asn Tyr Thr Ser Ser Leu Asn Leu Pro Asp Lys Thr Leu Gln Phe
340 345 350

Val Lys Asp His Pro Leu Met Asp Asp Ser Val Thr Pro Ile Asp Asn
355 360 365

Arg Pro Arg Leu Ile Lys Lys Asp Val Asn Tyr Thr Gln Ile Val Val
370 375 380

Asp Arg Thr Gln Ala Leu Asp Gly Thr Val Tyr Asp Val Met Phe Val
385 390 395 400

Ser Thr Asp Arg Gly Ala Leu His Lys Ala Ile Ser Leu Glu His Ala
405 410 415

Val His Ile Ile Glu Glu Thr Gln Leu Phe Gln Asp Phe Glu Pro Val
420 425 430

Gln Thr Leu Leu Leu Ser Ser Lys Lys Gly Asn Arg Phe Val Tyr Ala
435 440 445

Gly Ser Asn Ser Gly Val Val Gln Ala Pro Leu Ala Phe Cys Gly Lys
450 455 460

His Gly Thr Cys Glu Asp Cys Val Leu Ala Arg Asp Pro Tyr Cys Ala
465 470 475 480

Trp Ser Pro Pro Thr Ala Thr Cys Val Ala Leu His Gln Thr Glu Ser
485 490 495

Pro Ser Arg Gly Leu Ile Gln Glu Met Ser Gly Asp Ala Ser Val Cys
500 505 510

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Asp Lys Ser Lys Glu Ser Phe Asn Gln His Phe Phe Lys His Gly Gly
1 5 10 15

Thr Ala Glu Leu Lys Cys Phe Gln Lys Ser Asn Leu Ala Arg Val Val
20 25 30

Trp Lys Phe Gln Asn Gly Glu Leu Lys Ala Ala Ser Pro Lys Tyr Gly
35 40 45

Phe Val Gly Arg Lys His Leu Leu Ile Phe Asn Leu Ser Asp Gly Asp
50 55 60

Ser Gly Val Tyr Gln Cys Leu Ser Glu Glu Arg Val
65 70 75

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Asp Lys Ser Lys Gly Ser Tyr Arg Gln His Phe Phe Lys His Gly Gly
1 5 10 15

Thr Ala Glu Leu Lys Cys Ser Gln Lys Ser Asn Leu Ala Arg Val Phe
20 25 30

Trp Lys Phe Gln Asn Gly Val Leu Lys Ala Glu Ser Pro Lys Tyr Gly
35 40 45

Leu Met Gly Arg Lys Asn Leu Leu Ile Phe Asn Leu Ser Glu Gly Asp
50 55 60

Ser Gly Val Tyr Gln Cys Leu Ser Glu Glu Arg Val
65 70 75

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Arg Asn Lys Thr Val Ser Gln Leu Leu Ala Lys His Val Leu Glu Val
1               5                  10                  15

Lys Met Val Pro Arg Thr Pro Pro Ser Pro Thr Ser Glu Asp Ala Gln
            20                  25                  30

Thr Glu Gly Ser Lys Ile Thr Ser Lys Met Pro Val Ala Ser Thr Gln
        35                  40                  45

Gly Ser Ser Pro Pro Thr Pro Ala Leu Trp Ala Thr Ser Pro Arg Ala
    50                  55                  60

Ala Thr Leu Pro Pro Lys Ser Ser Ser Gly Thr Ser Cys Glu Pro Lys
65                  70                  75                  80

Met Val Ile Asn Thr Val Pro Gln Leu His Ser Glu Lys Thr Val Tyr
                85                  90                  95

Leu Lys Ser Ser Asp Asn
            100
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 103 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Lys Asn Lys Thr Val Phe Gln Val Val Ala Lys His Val Leu Glu Val
1               5                  10                  15

Lys Val Val Pro Lys Pro Val Val Ala Pro Thr Leu Ser Val Val Gln
            20                  25                  30

Thr Glu Gly Ser Arg Ile Ala Thr Lys Val Leu Val Ala Ser Thr Gln
        35                  40                  45

Gly Ser Ser Pro Pro Thr Pro Ala Val Gln Ala Thr Ser Ser Gly Ala
    50                  55                  60

Ile Thr Leu Pro Pro Lys Pro Ala Pro Thr Gly Thr Ser Cys Glu Pro
65                  70                  75                  80
```

Lys Ile Val Ile Asn Thr Val Pro Gln Leu His Ser Glu Lys Thr Met
85 90 95

Tyr Leu Lys Ser Ser Asp Asn
100

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Leu Leu Met Ser Leu Leu Leu Phe Ile Phe Val Leu Phe Leu Cys Leu
1 5 10 15

Phe Ser Tyr

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Leu Leu Met Ser Leu Phe Leu Phe Phe Phe Val Leu Phe Leu Cys Leu
1 5 10 15

Phe Phe Tyr

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Asn Cys Tyr Lys Gly Tyr Leu Pro Gly Gln Cys Leu Lys Phe Arg Ser
1 5 10 15

Ala Leu Leu Leu Gly Lys Lys Thr Pro Lys Ser Asp Phe Ser Asp Leu
20 25 30

Glu Gln Ser Val Lys Glu Thr Leu Val Glu Pro Gly Ser Phe Ser Gln
35 40 45

Gln Asn Gly Asp His Pro Lys Pro Ala Leu Asp Thr Gly Tyr Glu Thr
50 55 60

Glu Gln Asp Thr Ile Thr Ser Lys Val Pro Thr Asp Arg Glu Asp Ser
65 70 75 80

Gln Arg Ile Asp Glu Leu Ser Ala Arg Asp Lys Pro Phe Asp Val Lys
85 90 95

Cys Glu Leu Lys Phe Ala Asp Ser Asp Ala Asp Gly Asp
100 105

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Asn Cys Tyr Lys Gly Tyr Leu Pro Arg Gln Cys Leu Lys Phe Arg Ser
1 5 10 15

Ala Leu Leu Ile Gly Lys Lys Lys Pro Lys Ser Asp Phe Cys Asp Arg
20 25 30

Glu Gln Ser Leu Lys Glu Thr Leu Val Glu Pro Gly Ser Phe Ser Gln
35 40 45

Gln Asn Gly Glu His Pro Lys Pro Ala Leu Asp Thr Gly Tyr Glu Thr
50 55 60

Glu Gln Asp Thr Ile Thr Ser Lys Val Pro Thr Asp Arg Glu Asp Ser
65 70 75 80

Gln Arg Ile Asp Asp Leu Ser Ala Arg Asp Lys Pro Phe Asp Val Lys
85 90 95

Cys Glu Leu Lys Phe Ala Asp Ser Asp Ala Asp Gly Asp
100 105

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2589 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2586

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
ATG AGG ATG TGC ACC CCC ATT AGG GGG CTG CTC ATG GCC CTT GCA GTG          48
Met Arg Met Cys Thr Pro Ile Arg Gly Leu Leu Met Ala Leu Ala Val
  1               5                  10                  15

ATG TTT GGG ACA GCG ATG GCA TTT GCA CCC ATA CCC CGG ATC ACC TGG          96
Met Phe Gly Thr Ala Met Ala Phe Ala Pro Ile Pro Arg Ile Thr Trp
            20                  25                  30

GAG CAC AGA GAG GTG CAC CTG GTG CAG TTT CAT GAG CCA GAC ATC TAC         144
Glu His Arg Glu Val His Leu Val Gln Phe His Glu Pro Asp Ile Tyr
        35                  40                  45

AAC TAC TCA GCC TTG CTG CTG AGC GAG GAC AAG GAC ACC TTG TAC ATA         192
Asn Tyr Ser Ala Leu Leu Leu Ser Glu Asp Lys Asp Thr Leu Tyr Ile
    50                  55                  60

GGT GCC CGG GAG GCG GTC TTC GCT GTG AAC GCA CTC AAC ATC TCC GAG         240
Gly Ala Arg Glu Ala Val Phe Ala Val Asn Ala Leu Asn Ile Ser Glu
65                  70                  75                  80

AAG CAG CAT GAG GTG TAT TGG AAG GTC TCA GAA GAC AAA AAA GCA AAA         288
Lys Gln His Glu Val Tyr Trp Lys Val Ser Glu Asp Lys Lys Ala Lys
                85                  90                  95

TGT GCA GAA AAG GGG AAA TCA AAA CAG ACA GAG TGC CTC AAC TAC ATC         336
Cys Ala Glu Lys Gly Lys Ser Lys Gln Thr Glu Cys Leu Asn Tyr Ile
            100                 105                 110

CGG GTG CTG CAG CCA CTC AGC GCC ACT TCC CTT TAC GTG TGT GGG ACC         384
Arg Val Leu Gln Pro Leu Ser Ala Thr Ser Leu Tyr Val Cys Gly Thr
        115                 120                 125
```

```
AAC GCA TTC CAG CCG GCC TGT GAC CAC CTG AAC TTA ACA TCC TTT AAG          432
Asn Ala Phe Gln Pro Ala Cys Asp His Leu Asn Leu Thr Ser Phe Lys
    130                 135                 140

TTT CTG GGG AAA AAT GAA GAT GGC AAA GGA AGA TGT CCC TTT GAC CCA          480
Phe Leu Gly Lys Asn Glu Asp Gly Lys Gly Arg Cys Pro Phe Asp Pro
145                 150                 155                 160

GCA CAC AGC TAC ACA TCC GTC ATG GTT GAT GGA GAA CTT TAT TCG GGG          528
Ala His Ser Tyr Thr Ser Val Met Val Asp Gly Glu Leu Tyr Ser Gly
                165                 170                 175

ACG TCG TAT AAT TTT TTG GGA AGT GAA CCC ATC ATC TCC CGA AAT TCT          576
Thr Ser Tyr Asn Phe Leu Gly Ser Glu Pro Ile Ile Ser Arg Asn Ser
            180                 185                 190

TCC CAC AGT CCT CTG AGG ACA GAA TAT GCA ATC CCT TGG CTG AAC GAG          624
Ser His Ser Pro Leu Arg Thr Glu Tyr Ala Ile Pro Trp Leu Asn Glu
        195                 200                 205

CCT AGT TTC GTG TTT GCT GAC GTG ATC CGA AAA AGC CCA GAC AGC CCC          672
Pro Ser Phe Val Phe Ala Asp Val Ile Arg Lys Ser Pro Asp Ser Pro
    210                 215                 220

GAC GGC GAG GAT GAC AGG GTC TAC TTC TTC TTC ACG GAG GTG TCT GTG          720
Asp Gly Glu Asp Asp Arg Val Tyr Phe Phe Phe Thr Glu Val Ser Val
225                 230                 235                 240

GAG TAT GAG TTT GTG TTC AGG GTG CTG ATC CCA CGG ATA GCA AGA GTG          768
Glu Tyr Glu Phe Val Phe Arg Val Leu Ile Pro Arg Ile Ala Arg Val
                245                 250                 255

TGC AAG GGG GAC CAG GGC GGC CTG AGG ACC TTG CAG AAG AAA TGG ACC          816
Cys Lys Gly Asp Gln Gly Gly Leu Arg Thr Leu Gln Lys Lys Trp Thr
            260                 265                 270

TCC TTC CTG AAA GCC CGA CTC ATC TGC TCC CGG CCA GAC AGC GGC TTG          864
Ser Phe Leu Lys Ala Arg Leu Ile Cys Ser Arg Pro Asp Ser Gly Leu
        275                 280                 285

GTC TTC AAT GTG CTG CGG GAT GTC TTC GTG CTC AGG TCC CCG GGC CTG          912
Val Phe Asn Val Leu Arg Asp Val Phe Val Leu Arg Ser Pro Gly Leu
    290                 295                 300

AAG GTG CCT GTG TTC TAT GCA CTC TTC ACC CCA CAG CTG AAC AAC GTG          960
Lys Val Pro Val Phe Tyr Ala Leu Phe Thr Pro Gln Leu Asn Asn Val
305                 310                 315                 320

GGG CTG TCG GCA GTG TGC GCC TAC AAC CTG TCC ACA GCC GAG GAG GTC         1008
Gly Leu Ser Ala Val Cys Ala Tyr Asn Leu Ser Thr Ala Glu Glu Val
                325                 330                 335

TTC TCC CAC GGG AAG TAC ATG CAG AGC ACC ACA GTG GAG CAG TCC CAC         1056
Phe Ser His Gly Lys Tyr Met Gln Ser Thr Thr Val Glu Gln Ser His
            340                 345                 350
```

```
ACC AAG TGG GTG CGC TAT AAT GGC CCG GTA CCC AAG CCG CGG CCT GGA      1104
Thr Lys Trp Val Arg Tyr Asn Gly Pro Val Pro Lys Pro Arg Pro Gly
        355                 360                 365

GCG TGC ATC GAC AGC GAG GCA CGG GCC GCC AAC TAC ACC AGC TCC TTG      1152
Ala Cys Ile Asp Ser Glu Ala Arg Ala Ala Asn Tyr Thr Ser Ser Leu
    370                 375                 380

AAT TTG CCA GAC AAG ACG CTG CAG TTC GTT AAA GAC CAC CCT TTG ATG      1200
Asn Leu Pro Asp Lys Thr Leu Gln Phe Val Lys Asp His Pro Leu Met
385                 390                 395                 400

GAT GAC TCG GTA ACC CCA ATA GAC AAC AGG CCC AGG TTA ATC AAG AAA      1248
Asp Asp Ser Val Thr Pro Ile Asp Asn Arg Pro Arg Leu Ile Lys Lys
                405                 410                 415

GAT GTG AAC TAC ACC CAG ATC GTG GTG GAC CGG ACC CAG GCC CTG GAT      1296
Asp Val Asn Tyr Thr Gln Ile Val Val Asp Arg Thr Gln Ala Leu Asp
            420                 425                 430

GGG ACT GTC TAT GAT GTC ATG TTT GTC AGC ACA GAC CGG GGA GCT CTG      1344
Gly Thr Val Tyr Asp Val Met Phe Val Ser Thr Asp Arg Gly Ala Leu
        435                 440                 445

CAC AAA GCC ATC AGC CTC GAG CAC GCT GTT CAC ATC ATC GAG GAG ACC      1392
His Lys Ala Ile Ser Leu Glu His Ala Val His Ile Ile Glu Glu Thr
    450                 455                 460

CAG CTC TTC CAG GAC TTT GAG CCA GTC CAG ACC CTG CTG CTG TCT TCA      1440
Gln Leu Phe Gln Asp Phe Glu Pro Val Gln Thr Leu Leu Leu Ser Ser
465                 470                 475                 480

AAG AAG GGC AAC AGG TTT GTC TAT GCT GGC TCT AAC TCG GGC GTG GTC      1488
Lys Lys Gly Asn Arg Phe Val Tyr Ala Gly Ser Asn Ser Gly Val Val
                485                 490                 495

CAG GCC CCG CTG GCC TTC TGT GGG AAG CAC GGC ACC TGC GAG GAC TGT      1536
Gln Ala Pro Leu Ala Phe Cys Gly Lys His Gly Thr Cys Glu Asp Cys
            500                 505                 510

GTG CTG GCG CGG GAC CCC TAC TGC GCC TGG AGC CCG CCC ACA GCG ACC      1584
Val Leu Ala Arg Asp Pro Tyr Cys Ala Trp Ser Pro Pro Thr Ala Thr
        515                 520                 525

TGC GTG GCT CTG CAC CAG ACC GAG AGC CCC AGC AGG GGT TTG ATT CAG      1632
Cys Val Ala Leu His Gln Thr Glu Ser Pro Ser Arg Gly Leu Ile Gln
    530                 535                 540

GAG ATG AGC GGC GAT GCT TCT GTG TGC CCG GAT AAA AGT AAA GGA AGT      1680
Glu Met Ser Gly Asp Ala Ser Val Cys Pro Asp Lys Ser Lys Gly Ser
545                 550                 555                 560

TAC CGG CAG CAT TTT TTC AAG CAC GGT GGC ACA GCG GAA CTG AAA TGC      1728
Tyr Arg Gln His Phe Phe Lys His Gly Gly Thr Ala Glu Leu Lys Cys
                565                 570                 575
```

```
TCC CAA AAA TCC AAC CTG GCC CGG GTC TTT TGG AAG TTC CAG AAT GGC      1776
Ser Gln Lys Ser Asn Leu Ala Arg Val Phe Trp Lys Phe Gln Asn Gly
            580                 585                 590

GTG TTG AAG GCC GAG AGC CCC AAG TAC GGT CTT ATG GGC AGA AAA AAC      1824
Val Leu Lys Ala Glu Ser Pro Lys Tyr Gly Leu Met Gly Arg Lys Asn
        595                 600                 605

TTG CTC ATC TTC AAC TTG TCA GAA GGA GAC AGT GGG GTG TAC CAG TGC      1872
Leu Leu Ile Phe Asn Leu Ser Glu Gly Asp Ser Gly Val Tyr Gln Cys
    610                 615                 620

CTG TCA GAG GAG AGG GTT AAG AAC AAA ACG GTC TTC CAA GTG GTC GCC      1920
Leu Ser Glu Glu Arg Val Lys Asn Lys Thr Val Phe Gln Val Val Ala
625                 630                 635                 640

AAG CAC GTC CTG GAA GTG AAG GTG GTT CCA AAG CCC GTA GTG GCC CCC      1968
Lys His Val Leu Glu Val Lys Val Val Pro Lys Pro Val Val Ala Pro
                645                 650                 655

ACC TTG TCA GTT GTT CAG ACA GAA GGT AGT AGG ATT GCC ACC AAA GTG      2016
Thr Leu Ser Val Val Gln Thr Glu Gly Ser Arg Ile Ala Thr Lys Val
            660                 665                 670

TTG GTG GCA TCC ACC CAA GGG TCT TCT CCC CCA ACC CCA GCC GTG CAG      2064
Leu Val Ala Ser Thr Gln Gly Ser Ser Pro Pro Thr Pro Ala Val Gln
        675                 680                 685

GCC ACC TCC TCC GGG GCC ATC ACC CTT CCT CCC AAG CCT GCG CCC ACC      2112
Ala Thr Ser Ser Gly Ala Ile Thr Leu Pro Pro Lys Pro Ala Pro Thr
    690                 695                 700

GGC ACA TCC TGC GAA CCA AAG ATC GTC ATC AAC ACG GTC CCC CAG CTC      2160
Gly Thr Ser Cys Glu Pro Lys Ile Val Ile Asn Thr Val Pro Gln Leu
705                 710                 715                 720

CAC TCG GAG AAA ACC ATG TAT CTT AAG TCC AGC GAC AAC CGC CTC CTC      2208
His Ser Glu Lys Thr Met Tyr Leu Lys Ser Ser Asp Asn Arg Leu Leu
                725                 730                 735

ATG TCC CTC TTC CTC TTC TTC TTT GTT CTC TTC CTC TGC CTC TTT TTC      2256
Met Ser Leu Phe Leu Phe Phe Phe Val Leu Phe Leu Cys Leu Phe Phe
            740                 745                 750

TAC AAC TGC TAT AAG GGA TAC CTG CCC AGA CAG TGC TTG AAA TTC CGC      2304
Tyr Asn Cys Tyr Lys Gly Tyr Leu Pro Arg Gln Cys Leu Lys Phe Arg
        755                 760                 765

TCG GCC CTA CTA ATT GGG AAG AAG AAG CCC AAG TCA GAT TTC TGT GAC      2352
Ser Ala Leu Leu Ile Gly Lys Lys Lys Pro Lys Ser Asp Phe Cys Asp
    770                 775                 780

CGT GAG CAG AGC CTG AAG GAG ACG TTA GTA GAG CCA GGG AGC TTC TCC      2400
Arg Glu Gln Ser Leu Lys Glu Thr Leu Val Glu Pro Gly Ser Phe Ser
785                 790                 795                 800
```

```
CAG CAG AAT GGG GAG CAC CCC AAG CCA GCC CTG GAC ACC GGC TAT GAG      2448
Gln Gln Asn Gly Glu His Pro Lys Pro Ala Leu Asp Thr Gly Tyr Glu
                805                 810                 815

ACC GAG CAA GAC ACC ATC ACC AGC AAA GTC CCC ACG GAT AGG GAG GAC      2496
Thr Glu Gln Asp Thr Ile Thr Ser Lys Val Pro Thr Asp Arg Glu Asp
            820                 825                 830

TCA CAG AGG ATC GAC GAC CTT TCT GCC AGG GAC AAG CCC TTT GAC GTC      2544
Ser Gln Arg Ile Asp Asp Leu Ser Ala Arg Asp Lys Pro Phe Asp Val
        835                 840                 845

AAG TGT GAG CTG AAG TTC GCT GAC TCA GAC GCA GAT GGA GAC              2586
Lys Cys Glu Leu Lys Phe Ala Asp Ser Asp Ala Asp Gly Asp
    850                 855                 860

TGA                                                                 2589
 *
```

CLAIMS

1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a CD100 antigen.

2. The isolated nucleic acid molecule of claim 1, which is a cDNA.

3. The isolated nucleic acid molecule of claim 2, wherein the CD100 antigen is human.

4. The isolated nucleic acid of claim 3 comprising a nucleotide sequence shown in Figure 1, SEQ ID NO: 1.

5. The isolated nucleic acid of claim 4 comprising the coding region.

6. The isolated nucleic acid of claim 3 derived from a hematopoietic cell which hybridizes under high stringency conditions to a nucleic acid molecule comprising a nucleotide sequence shown in Figure 1, SEQ ID NO: 1.

7. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein wherein the protein comprises an amino acid sequence having at least about 80% overall amino acid sequence identity with an amino acid sequence shown in Figure 2, SEQ ID NO: 2.

8. The isolated nucleic acid molecule of claim 7, wherein the protein has a CD100 activity.

9. An isolated nucleic acid molecule encoding an amino acid sequence shown in Figure 2, SEQ ID NO: 2.

10. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein, wherein the protein comprises a semaphorin domain having an amino acid sequence at least 80% identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2.

11. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein, wherein the protein comprises

a) a semaphorin domain having an amino acid sequence at least 60% identical to an amino acid sequence shown in Figure 2,

SEQ ID NO: 2; and

b) an immunoglobulin-like domain having an amino acid sequence at least 50% identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2.

12. The isolated nucleic acid molecule of claim 11, further comprising

c) a cytoplasmic domain having an amino acid sequence at least 50% identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2.

13. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein, wherein the protein comprises an extracellular domain having an amino acid sequence at least 80% identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2.

14. An isolated nucleic acid molecule encoding a CD100 fusion protein comprising a nucleotide sequence encoding a first peptide having an amino acid sequence at least 80% identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2 and a nucleic sequence encoding a second peptide corresponding to a moiety that alters the solubility, binding affinity or valency of the first peptide.

15. The isolated nucleic acid molecule of claim 14, wherein the first peptide comprises an extracellular domain of a human CD100 antigen.

16. The isolated nucleic acid molecule of claim 14, wherein the first peptide comprises a semaphorin domain of a human CD100 antigen.

17. The isolated nucleic acid molecule of claim 14, wherein the second peptide comprises an immunoglobulin constant region.

18. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a peptide comprising a fragment of at least about 30 amino acids of the sequence shown in Figure 2, SEQ ID NO: 2.

19. The isolated nucleic acid molecule of claim 18, wherein the peptide has a CD100 activity.

20. An isolated nucleic acid molecule of claim 1, which is antisense to the nucleic acid molecule of claim 1.

21. An isolated nucleic acid molecule of claim 1, which is antisense to the nucleic acid molecule of claim 4.

31. An isolated nucleic acid molecule of claim 1, which is antisense to the nucleic acid molecule of claim 5.

32. A vector comprising a nucleotide sequence encoding a CD100 antigen.

33. A vector comprising a nucleotide sequence encoding a protein comprising an amino acid sequence shown in Figure 2, SEQ ID NO: 2.

34. A host cell comprising the vector of claim 32.

35. A host cell comprising the vector of claim 33.

36. A method for producing a CD100 antigen comprising culturing a host cell of claim 34 in a suitable medium such that the CD100 antigen is produced.

37. A method for producing a CD100 antigen comprising culturing a host cell of claim 35 in a suitable medium such that the CD100 antigen is produced.

38. An isolated protein having a CD100 activity.

39. The isolated protein of claim 38, which is human.

40. The isolated protein of claim 39, wherein the protein comprises an amino acid sequence having at least about 80% overall amino acid sequence identity with an amino acid sequence shown in Figure 2 (SEQ ID NO: 2).

41. The isolated protein of claim 40, wherein the protein comprises an amino acid sequence having at least about 90% overall amino acid sequence identity with an amino acid sequence shown in Figure 2 (SEQ ID NO: 2).

42. The isolated protein of claim 40, wherein the protein comprises an amino acid sequence having at least about 95% overall amino acid sequence identity with an amino acid sequence shown in Figure 2 (SEQ ID NO: 2).

43. The isolated protein of claim 39, wherein the protein has a CD100 activity.

44. The isolated protein of claim 43, which comprises an amino acid sequence shown in Figure 2 (SEQ ID NO: 2).

45. An isolated protein comprising a semaphorin domain having an amino acid sequence at least 80% identical to an amino acid sequence shown in Figure 2 (SEQ ID NO: 2).

46. The isolated protein of claim 45 having an amino acid sequence at least 90% identical to an amino acid sequence shown in Figure 2 (SEQ ID NO: 2).

47. An isolated protein comprising

a) a semaphorin domain having an amino acid sequence at least 60% identical to an amino acid sequence shown in Figure 2 (SEQ ID NO: 2); and

b) an immunoglobulin-like domain having an amino acid sequence that is at least 50% identical to an amino acid sequence shown in Figure 2 (SEQ ID NO: 2).

48. The isolated protein of claim 47, further comprising

c) a cytoplasmic domain having an amino acid sequence at least 50% identical to an amino acid sequence shown in Figure 2 (SEQ ID NO: 2).

49. An isolated protein comprising an extracellular domain having an amino acid sequence at least 80% identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2.

50. An CD100 fusion protein comprising a first peptide having an amino acid sequence at least 80% identical to an amino acid sequence shown in Figure 2, SEQ ID NO: 2 and a second peptide corresponding to a moiety that alters the solubility, binding affinity or valency of the first peptide.

51. The fusion protein of claim 50, wherein the first peptide comprises an extracellular domain of a human CD100 antigen.

52. The fusion protein of claim 50, wherein the first peptide comprises a semaphorin domain of a human CD100 antigen.

53. The fusion protein of claim 50, wherein the second peptide comprises an immunoglobulin constant region.

54. A peptide comprising a fragment of at least about 30 amino acids of the sequence shown in Figure 2, SEQ ID NO: 2.

55. The peptide of claim 54, which has a CD100 activity.

56. The peptide of claim 54, wherein the fragment is at least about 40 amino acids in length.

57. The peptide of claim 56, which has a CD100 activity.

58. The peptide of claim 56, wherein the fragment is at least about 50 amino acid in length.

59. The peptide of claim 58, which has a CD100 activity.

60. A pharmaceutical composition comprising the protein of claim 44 and a pharmaceutically acceptable carrier.

61. A pharmaceutical composition comprising the protein of claim 50 and a pharmaceutically acceptable carrier.

62. A pharmaceutical composition comprising the protein of claim 55 and a pharmaceutically acceptable carrier.

63. A vaccine composition comprising at least one antigen and a first agent which stimulates a CD100 ligand-associated signal.

64. The composition of claim 63, further comprising a second agent which stimulates a CD40-associated signal.

65. A method for modulating a B cell response comprising contacting the B cell with an agent which modulates a CD100 ligand-associated signal in the B cell, such that a response by the B cell is modulated.

66. The method of claim 65, wherein the agent stimulates a CD100 ligand associated signal, such that a response by the B cell is stimulated.

67. The method of claim 66, wherein the agent is a stimulatory form of a CD100 antigen.

68. The method of claim 67, wherein the stimulatory form of a CD100 antigen is a soluble form of a CD100 antigen.

69. The method of claim 68, wherein the soluble form of a CD100 antigen is an immunoglobulin fusion protein.

70. The method of claim 67, wherein the stimulatory form of a CD100 antigen is attached to a solid phase support.

71. The method of claim 68, wherein the solid phase support is a cell membrane.

72. The method of claim 66, further comprising contacting the B cell with a second agent which provides a stimulatory signal to the B cell.

73. The method of claim 72, wherein the second agent stimulates a CD40 associated signal in the B cell.

74. The method of claim 73, wherein the second agent is a CD40 ligand.

75. The method of claim 66, further comprising contacting the B cells with T cells.

76. The method of claim 75, further comprising contacting the B cells with at least one antigen.

77. The method of claim 65, wherein the agent inhibits a CD100 ligand-associated signal, such that a response by the B cell is inhibited.

78. The method of claim 77, wherein the agent interacts with CD100.

79. The method of claim 78, wherein the agent is an antibody to CD100.

80. The method of claim 77, wherein the agent interacts with a CD100 ligand.

81. The method of claim 80, wherein the agent is an inhibitory form of CD100.

82. The method of claim 65, wherein the B cell response is B cell aggregation.

83. The method of claim 65, wherein the B cell response is B cell differentiation.

84. The method of claim 83, wherein the B cells differentiate into plasma cells.

85. The method of claim 83, wherein the B cells differentiate to memory B cells.

86. The method of claim 65, wherein the B cell response is B cell viability.

87. The method of claim 65, wherein contacting the cells is in a subject, such that a response by the B cell is modulated in the subject.

88. The method of claim 87, wherein the agent stimulates a B cell response in a subject infected with a pathogen, such that elimination of the pathogen by the subject is enhanced.

89. The method of claim 87, wherein the agent stimulates a B cell response in a subject having a low grade lymphoma, such that the B cell differentiation in the subject is stimulated.

90. The method of claim 88, further comprising administering to the subject a second agent which stimulates a CD40-associated signal in the B cell.

91. The method of claim 87, wherein the agent inhibits a B cell response in an allergic subject, such that the B cell response by the subject to an allergen is inhibited.

92. The method of claim 87, wherein the agent inhibits a B cell response in a subject having a large cell lymphoma, such that differentiation of the B cell in the subject is inhibited.

93. A method for modulating an interaction between an immune cell and a nerve cell in a subject, comprising administering to a subject an agent which modulates a CD100 ligand-associated signal in the nerve cell, such that modulation of the interaction between the immune cell and the nerve cell in the subject occurs.

94. A method for modulating axonal growth of a neuron, comprising contacting the neuron with a modulating form of CD100, such that axonal growth is modulated.

95. A method for modulating a T cell response, comprising contacting the T cell with an agent which modulates a CD100 ligand-induced signal in the T cell, such that a response in the T cell is modulated.

96. The method of claim 95, wherein a T cell is contacted with an agent which stimulates a CD100 ligand-induced signal in the T cell, such that a response in the T cell is stimulated.

97. The method of claim 96, further comprising contacting the T cell with an agent which provides a primary activation signal to the T cell.

98. The method of claim 95, wherein the T cell is contacted with an agent which inhibits a CD100 ligand-induced signal in the T cell, such that a response in the T cell is inhibited.

FIGURE 1

CD100 DNA and predicted protein sequence

```
1                                                                CTGAGCCGCATCTGCAATAG
21 CACACTTGCCCGGCCACCTGCTGCCGTGAGCCTTTGCTGCTGAAGCCCCTGGGGTCGCCTCTACCTG
   Met Arg Met Cys Thr Pro Ile Arg Gly Leu Leu Met Ala Leu Ala Val Met   17
88 ATG AGG ATG TGC ACC CCC ATT AGG GGG CTG CTC ATG GCC CTT GCA GTG ATG
   Phe Gly Thr Ala Met Ala Phe Ala Pro Ile Pro Arg Ile Thr Trp Glu His   34
139 TTT GGG ACA GCG ATG GCA TTT GCA CCC ATA CCC CGG ATC ACC TGG GAG CAC
   Arg Glu Val His Leu Val Gln Phe His Glu Pro Asp Ile Tyr Asn Tyr Ser   51
190 AGA GAG GTG CAC CTG GTG CAG TTT CAT GAG CCA GAC ATC TAC AAC TAC TCA
   Ala Leu Leu Leu Ser Glu Asp Lys Asp Thr Leu Tyr Ile Gly Ala Arg Glu   68
241 GCC TTG CTG CTG AGC GAG GAC AAG GAC ACC TTG TAC ATA GGT GCC CGG GAG
   Ala Val Phe Ala Val Asn Ala Leu Asn Ile Ser Glu Lys Gln His Glu Val   85
292 GCG GTC TTC GCT GTG AAC GCA CTC AAC ATC TCC GAG AAG CAG CAT GAG GTG
   Tyr Trp Lys Val Ser Glu Asp Lys Lys Ala Lys Cys Ala Glu Lys Gly Lys  102
343 TAT TGG AAG GTC TCA GAA GAC AAA AAA GCA AAA TGT GCA GAA AAG GGG AAA
   Ser Lys Gln Thr Glu Cys Leu Asn Tyr Ile Arg Val Leu Gln Pro Leu Ser  119
394 TCA AAA CAG ACA GAG TGC CTC AAC TAC ATC CGG GTG CTG CAG CCA CTC AGC
   Ala Thr Ser Leu Tyr Val Cys Gly Thr Asn Ala Phe Gln Pro Ala Cys Asp  136
445 GCC ACT TCC CTT TAC GTG TGT GGG ACC AAC GCA TTC CAG CCG GCC TGT GAC
   His Leu Asn Leu Thr Ser Phe Lys Phe Leu Gly Lys Asn Glu Asp Gly Lys  153
496 CAC CTG AAC TTA ACA TCC TTT AAG TTT CTG GGG AAA AAT GAA GAT GGC AAA
   Gly Arg Cys Pro Phe Asp Pro Ala His Ser Tyr Thr Ser Val Met Val Asp  170
547 GGA AGA TGT CCC TTT GAC CCA GCA CAC AGC TAC ACA TCC GTC ATG GTT GAT
   Gly Glu Leu Tyr Ser Gly Thr Ser Tyr Asn Phe Leu Gly Ser Glu Pro Ile  187
598 GGA GAA CTT TAT TCG GGG ACG TCG TAT AAT TTT TTG GGA AGT GAA CCC ATC
   Ile Ser Arg Asn Ser Ser His Ser Pro Leu Arg Thr Glu Tyr Ala Ile Pro  204
649 ATC TCC CGA AAT TCT TCC CAC AGT CCT CTG AGG ACA GAA TAT GCA ATC CCT
   Trp Leu Asn Glu Pro Ser Phe Val Phe Ala Asp Val Ile Arg Lys Ser Pro  221
700 TGG CTG AAC GAG CCT AGT TTC GTG TTT GCT GAC GTG ATC CGA AAA AGC CCA
   Asp Ser Pro Asp Gly Glu Asp Asp Arg Val Tyr Phe Phe Phe Thr Glu Val  238
751 GAC AGC CCC GAC GGC GAG GAT GAC AGG GTC TAC TTC TTC TTC ACG GAG GTG
   Ser Val Glu Tyr Glu Phe Val Phe Arg Val Leu Ile Pro Arg Ile Ala Arg  255
802 TCT GTG GAG TAT GAG TTT GTG TTC AGG GTG CTG ATC CCA CGG ATA GCA AGA
   Val Cys Lys Gly Asp Gln Gly Gly Leu Arg Thr Leu Gln Lys Lys Trp Thr  272
853 GTG TGC AAG GGG GAC CAG GGC GGC CTG AGG ACC TTG CAG AAG AAA TGG ACC
   Ser Phe Leu Lys Ala Arg Leu Ile Cys Ser Arg Pro Asp Ser Gly Leu Val  289
904 TCC TTC CTG AAA GCC CGA CTC ATC TGC TCC CGG CCA GAC AGC GGC TTG GTC
   Phe Asn Val Leu Arg Asp Val Phe Val Leu Arg Ser Pro Gly Leu Lys Val  306
955 TTC AAT GTG CTG CGG GAT GTC TTC GTG CTC AGG TCC CCG GGC CTG AAG GTG
   Pro Val Phe Tyr Ala Leu Phe Thr Pro Gln Leu Asn Asn Val Gly Leu Ser  323
1006 CCT GTG TTC TAT GCA CTC TTC ACC CCA CAG CTG AAC AAC GTG GGG CTG TCG
   Ala Val Cys Ala Tyr Asn Leu Ser Thr Ala Glu Glu Val Phe Ser His Gly  340
1057 GCA GTG TGC GCC TAC AAC CTG TCC ACA GCC GAG GAG GTC TTC TCC CAC GGG
   Lys Tyr Met Gln Ser Thr Thr Val Glu Gln Ser His Thr Lys Trp Val Arg  357
1108 AAG TAC ATG CAG AGC ACC ACA GTG GAG CAG TCC CAC ACC AAG TGG GTG CGC
   Tyr Asn Gly Pro Val Pro Lys Pro Arg Pro Gly Ala Cys Ile Asp Ser Glu  374
1159 TAT AAT GGC CCG GTA CCC AAG CCG CGG CCT GGA GCG TGC ATC GAC AGC GAG
   Ala Arg Ala Ala Asn Tyr Thr Ser Ser Leu Asn Leu Pro Asp Lys Thr Leu  391
1210 GCA CGG GCC GCC AAC TAC ACC AGC TCC TTG AAT TTG CCA GAC AAG ACG CTG
   Gln Phe Val Lys Asp His Pro Leu Met Asp Asp Ser Val Thr Pro Ile Asp  408
1261 CAG TTC GTT AAA GAC CAC CCT TTG ATG GAT GAC TCG GTA ACC CCA ATA GAC
   Asn Arg Pro Arg Leu Ile Lys Lys Asp Val Asn Tyr Thr Gln Ile Val Val  425
1312 AAC AGG CCC AGG TTA ATC AAG AAA GAT GTG AAC TAC ACT CAG ATC GTG GTG
   Asp Arg Thr Gln Ala Leu Asp Gly Thr Val Tyr Asp Val Met Phe Val Ser  442
1363 GAC CGG ACC CAG GCC CTG GAT GGG ACT GTC TAT GAT GTC ATG TTT GTC AGC
   Thr Asp Arg Gly Ala Leu His Lys Ala Ile Ser Leu Glu His Ala Val His  459
1414 ACA GAC CGG GGA GCT CTG CAC AAA GCC ATC AGC CTC GAG CAC GCT GTT CAC
   Ile Ile Glu Glu Thr Gln Leu Phe Gln Asp Phe Glu Pro Val Gln Thr Leu  476
1465 ATC ATC GAG GAG ACC CAG CTC TTC CAG GAC TTT GAG CCA GTC CAG ACC CTG
   Leu Leu Ser Ser Lys Lys Gly Asn Arg Phe Val Tyr Ala Gly Ser Asn Ser  493
1516 CTG CTG TCT TCA AAG AAG GGC AAC AGG TTT GTC TAT GCT GGC TCT AAC TCG
   Gly Val Val Gln Ala Pro Leu Ala Phe Cys Gly Lys His Gly Thr Cys Glu  510
1567 GGC GTG GTC CAG GCC CCG CTG GCC TTC TGT GGG AAG CAC GGC ACC TGC GAG
   Asp Cys Val Leu Ala Arg Asp Pro Tyr Cys Ala Trp Ser Pro Pro Thr Ala  527
1618 GAC TGT GTG CTG GCG CGG GAC CCC TAC TGC GCC TGG AGC CCG CCC ACA GCG
   Thr Cys Val Ala Leu His Gln Thr Glu Ser Pro Ser Arg Gly Leu Ile Gln  544
1669 ACC TGC GTG GCT CTG CAC CAG ACC GAG AGC CCC AGC AGG GGT TTG ATT CAG
   Glu Met Ser Gly Asp Ala Ser Val Cys Pro Asp Lys Ser Lys Gly Ser Tyr  561
1720 GAG ATG AGC GGC GAT GCT TCT GTG TGC CCG GAT AAA AGT AAA GGA AGT TAC
   Arg Gln His Phe Phe Lys His Gly Gly Thr Ala Glu Leu Lys Cys Ser Gln  578
1771 CGG CAG CAT TTT TTC AAG CAC GGT GGC ACA GCG GAA CTG AAA TGC TCC CAA
   Lys Ser Asn Leu Ala Arg Val Phe Trp Lys Phe Gln Asn Gly Val Leu Lys  595
1822 AAA TCC AAC CTG GCC CGG GTC TTT TGG AAG TTC CAG AAT GGC GTG TTG AAG
   Ala Glu Ser Pro Lys Tyr Gly Leu Met Gly Arg Lys Asn Leu Leu Ile Phe  612
1873 GCC GAG AGC CCC AAG TAC GGT CTT ATG GGC AGA AAA AAC TTG CTC ATC TTC
   Asn Leu Ser Glu Gly Asp Ser Gly Val Tyr Gln Cys Leu Ser Glu Glu Arg  629
1924 AAC TTG TCA GAA GGA GAC AGT GGG GTG TAC CAG TGC CTG TCA GAG GAG AGG
   Val Lys Asn Lys Thr Val Phe Gln Val Val Ala Lys His Val Leu Glu Val  646
1975 GTT AAG AAC AAA ACG GTC TTC CAA GTG GTC GCC AAG CAC GTC CTG GAA GTG
   Lys Val Val Pro Lys Pro Val Val Ala Pro Thr Leu Ser Val Val Gln Thr  663
2026 AAG GTG GTT CCA AAG CCC GTA GTG GCC CCC ACC TTG TCA GTT GTT CAG ACA
   Glu Gly Ser Arg Ile Ala Thr Lys Val Leu Val Ala Ser Thr Gln Gly Ser  680
```

FIGURE 1 (CONTINUED)

```
2077 GAA GGT AGT AGG ATT GCC ACC AAA GTG TTG GTG GCA TCC ACC CAA GGG TCT
     Ser Pro Pro Thr Pro Ala Val Gln Ala Thr Ser Ser Gly Ala Ile Thr Leu   697
2128 TCT CCC CCA ACC CCA GCC GTG CAG GCC ACC TCC TCC GGG GCC ATC ACC CTT
     Pro Pro Lys Pro Ala Pro Thr Gly Thr Ser Cys Glu Pro Lys Ile Val Ile   714
2179 CCT CCC AAG CCT GCG CCC ACC GGT ACA TCC TGC GAA CCA AAG ATC GTC ATC
     Asn Thr Val Pro Gln Leu His Ser Glu Lys Thr Met Tyr Leu Lys Ser Ser   731
2230 AAC ACG GTC CCC CAG CTC CAC TCG GAG AAA ACC ATG TAT CTT AAG TCC AGC
     Asp Asn Arg Leu Leu Met Ser Leu Phe Leu Phe Phe Phe Val Leu Phe Leu   748
2281 GAC AAC CGC CTC CTC ATG TCC CTC TTC CTC TTC TTC TTT GTT CTC TTC CTC
     Cys Leu Phe Phe Tyr Asn Cys Tyr Lys Gly Tyr Leu Pro Arg Gln Cys Leu   765
2332 TGC CTC TTT TTC TAC AAC TGC TAT AAG GGA TAC CTG CCC AGA CAG TGC TTG
     Lys Phe Arg Ser Ala Leu Leu Ile Gly Lys Lys Lys Pro Lys Ser Asp Phe   782
2383 AAA TTC CGC TCG GCC CTA CTA ATT GGG AAG AAG AAG CCC AAG TCA GAT TTC
     Cys Asp Arg Glu Gln Ser Leu Lys Glu Thr Leu Val Glu Pro Gly Ser Phe   799
2434 TGT GAC CGT GAG CAG AGC CTG AAG GAG ACG TTA GTA GAG CCA GGG AGC TTC
     Ser Gln Gln Asn Gly Glu His Pro Lys Pro Ala Leu Asp Thr Gly Tyr Glu   816
2485 TCC CAG CAG AAT GGG GAG CAC CCC AAG CCA GCC CTG GAC ACC GGC TAT GAG
     Thr Glu Gln Asp Thr Ile Thr Ser Lys Val Pro Thr Asp Arg Glu Asp Ser   833
2536 ACC GAG CAA GAC ACC ATC ACC AGC AAA GTC CCC ACG GAT AGG GAG GAC TCA
     Gln Arg Ile Asp Asp Leu Ser Ala Arg Asp Lys Pro Phe Asp Val Lys Cys   850
2587 CAG AGG ATC GAC GAC CTT TCT GCC AGG GAC AAG CCC TTT GAC GTC AAG TGT
     Glu Leu Lys Phe Ala Asp Ser Asp Ala Asp Gly Asp Stop                  862
2638 GAG CTG AAG TTC GCT GAC TCA GAC GCA GAT GGA GAC TGA GGCCGGCTGTGCATC
2692 CCCGCTGGTGCCTCGGCTGCGACGTGTCCAGGCGTGGAGAGTTTTGTGTTTCTCCTGTTCAGTATCC
2759 GAGTCTCGTGCAGTGCTGCGTAGGTTAGCCCGCATCGTGCAGACAACCTCAGTCCTCTTGTCTATTT
2826 TCTCTTGGGTTGAGCCTGTGACTTGGTTTCTCTTTGTCCTTTTGGAAAAATGACAAGCATTGCATCC
2893 CAGTCTTGTGTTCCGAAGTCAGTCGGAGTACTTGAAGAAGGCCCACGGGCGGCACGGAGTTCCTGAG
2960 CCCTTTCTGTAGTGGGGGAAAGGTGGCTGGACCTCTGTTGGCTGAGAAGAGCATCCCTTCAGCTTCC
3027 CCTCCCCGTAGCAGCCACTAAAAGATTATTTAATTCCAGATTGGAAATGACATTTTAGTTTATCAGA
3094 TTGGTAACTTATCGCCTGTTGTCCAGATTGGCACGAACCTTTTCTTCCACTTAATTATTTTTTTAGG
3161 ATTTTGCTTTGATTGTGTTTATGTCATGGGTCATTTTTTTTTAGTTACAGAAGCAGTTGTGTTAATA
3228 TTTAGAAGAAGATGTATATCTTTCCAGATTTTGTTATATATTTGGCATAAAATACGGCTTACGTTGCT
3295 TAAGATTCTCAGGGATAAACTTCCTTTTGCTAAATGCATTCTTTCTGCTTTTAGAAATGTAGACATA
3362 AACACTCCCCGGAGCCCACTCACCTTTTTTCTTTTTCTTTTTTTTTTTTTTAACTTTATTCCTTGAGG
3429 GAAGCATTGTTTTTGGAGAGATTTTCTTTCTGTACTTCGTTTTACTTTTCTTTTTTTTTAACTTTTA
3496 CTCTCTCGAAGAAGAGGACCTTCCCACATCCA GAGGTGGTTTTGAGCAAGGGAAGGTAGCCTGGA
3563 TGAGCTGAGTGGAGCCAGGCTGGTCCAGAGCTGAGATTTAGTGCGGTACAATCTGGAGCCCACAGC
3630 TGTCGGTCAGAACCTCCTGTGAGACAGATGAAACTTTCACAAGGCGAGTTTGGTTCTCTGAACATC
3697 TCCTTTCTCTTCTTGCTTCAATTGCTTACCCACTGCCTGCCCAGACTTTCTATCCAGCCTCACTGAG
3764 CTGCCCACTACTGGAAGGGAACTGGGCCTCGGTGGCCGGGCCCGAGCTGTGACCACAGCACCCTC
3831 AAGCATACGGCGCTGTTCCTGCCACTGTCCTGAAGATGTGAATGGGTGGTACGATTTCAACACTGGT
3898 TAATTTCACACTCCATCTCCCCGCTTTGTAAATAACCATGGGAAGAGACTTTTTTTCCATGGTGAA
3965 GAGCAATAAACTCTGGATGTTTGTGCGCGTGTGTGGACAGTCTTATCTTCCAGCATGATAGGATTTG
4032 ACCATTTTGGTGTAAACATTTGTGTTTTATAAGATTTACCTTGTTTTTATTTTTCTACTTTGAATTG
4099 TATACATTTGGAAAGTACCCAAATAAATGAGAAGCTTCTATCCTTAAAAAAAAAAAAAA
```

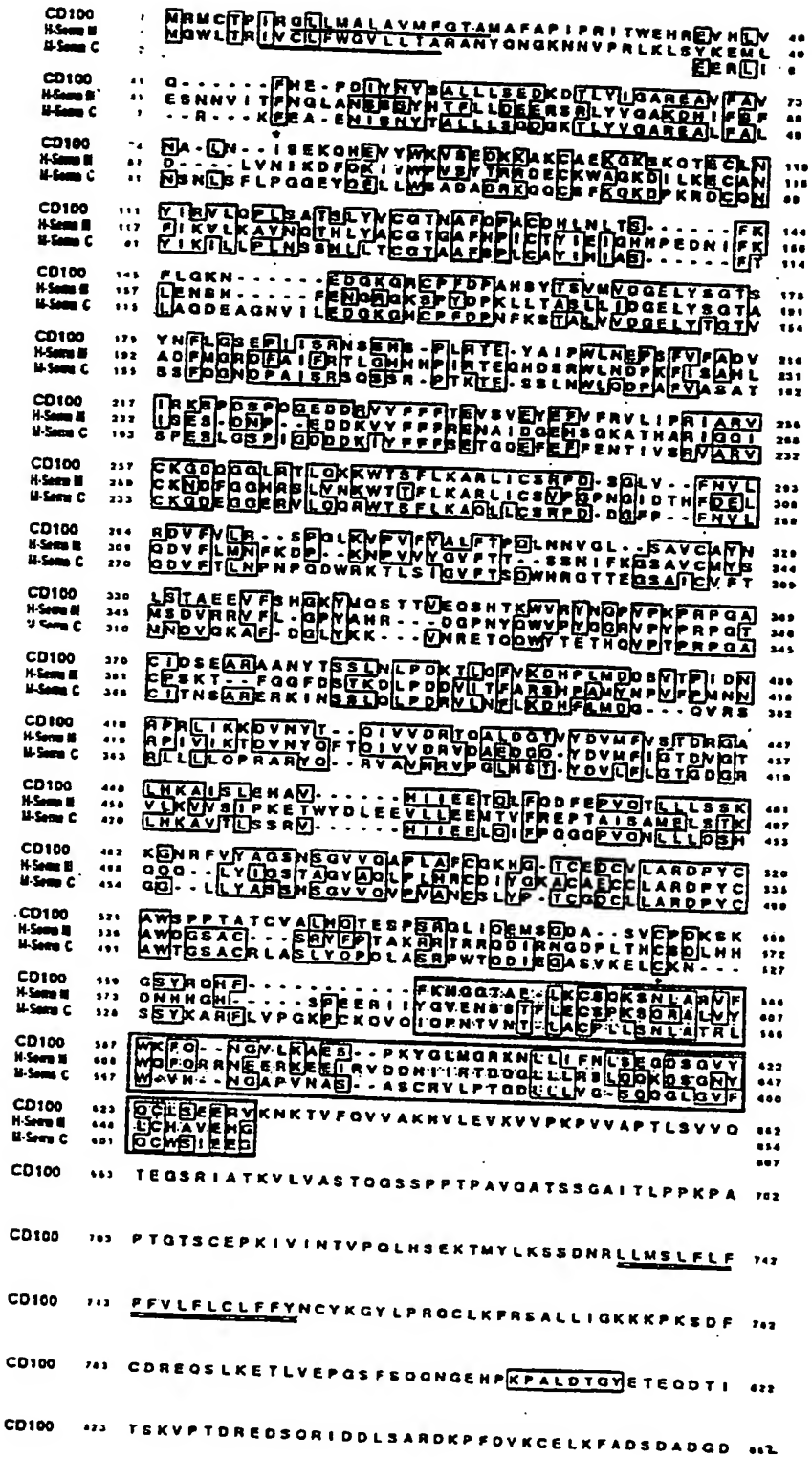


FIGURE 2

FIGURE 3

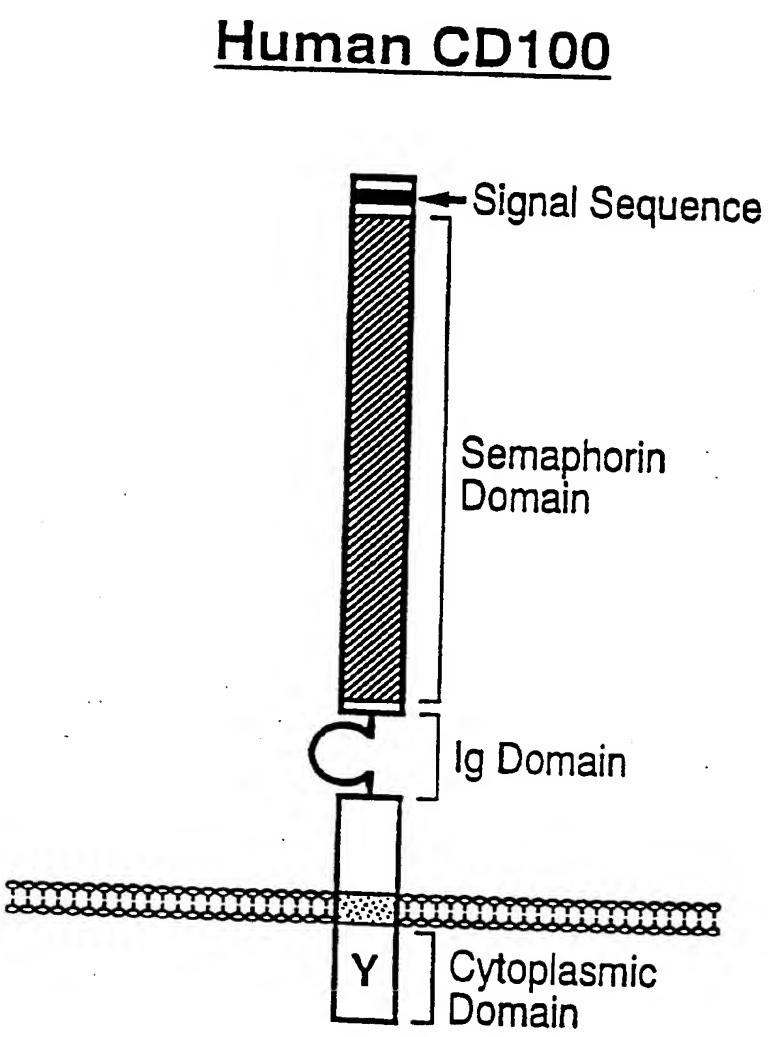


FIGURE 4

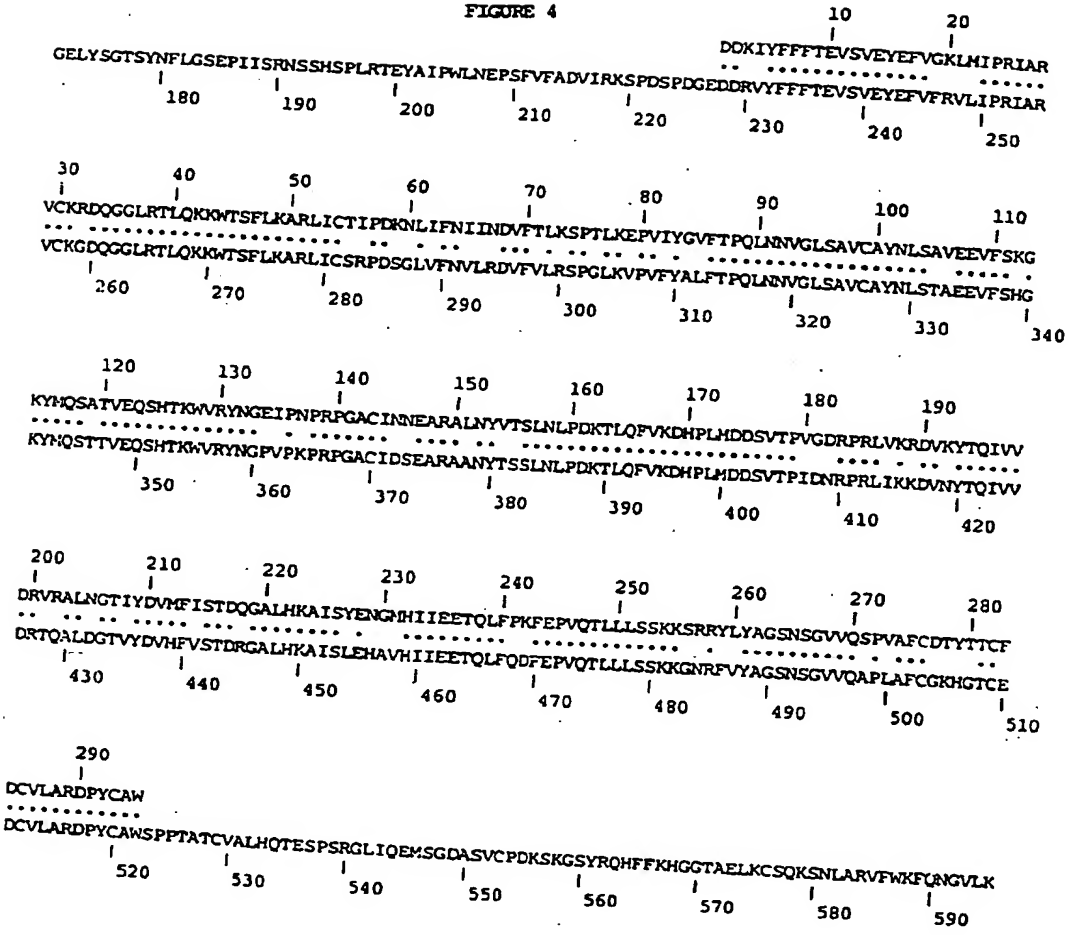


FIGURE 5

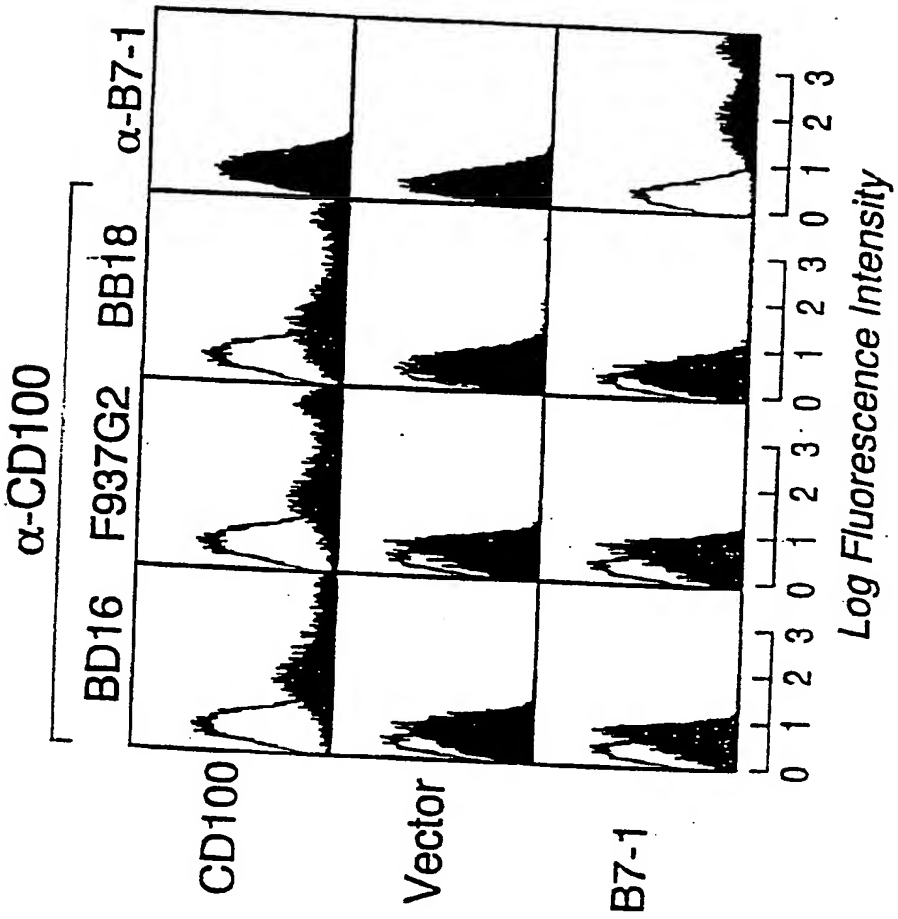


FIGURE 6

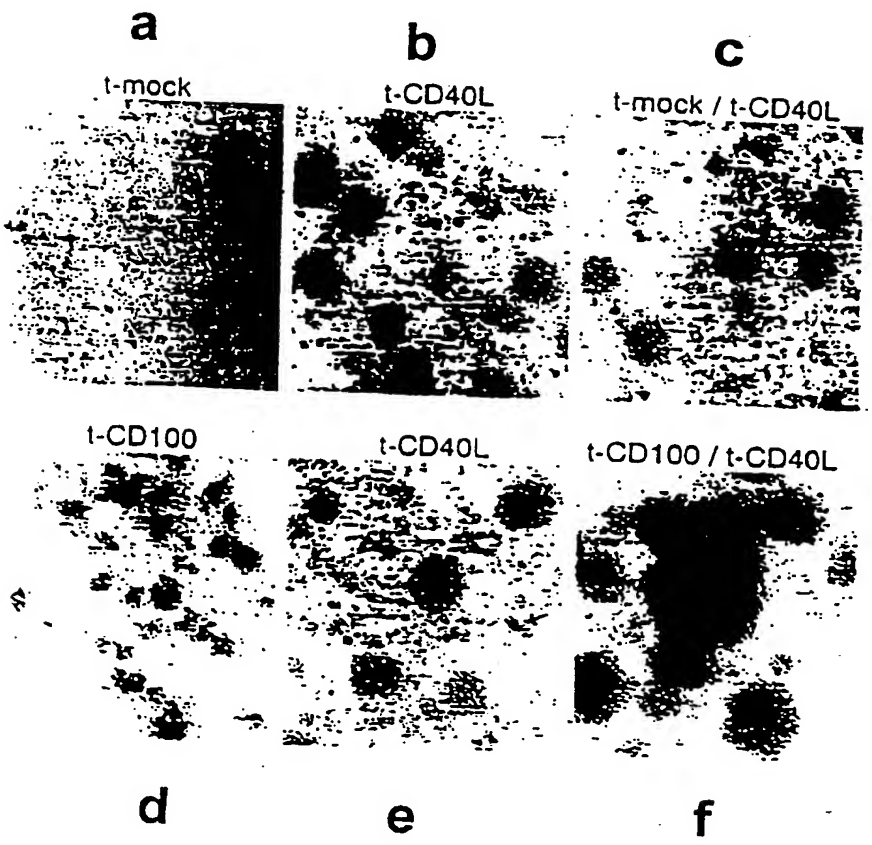


FIGURE 7

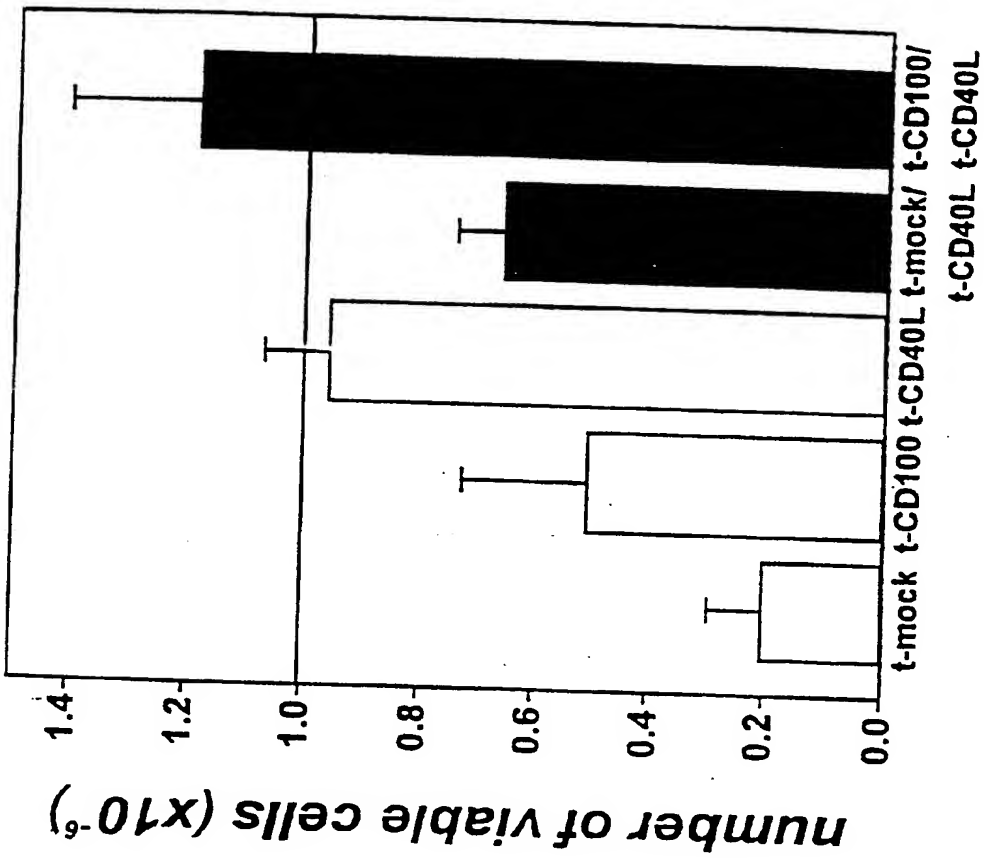


FIGURE 8

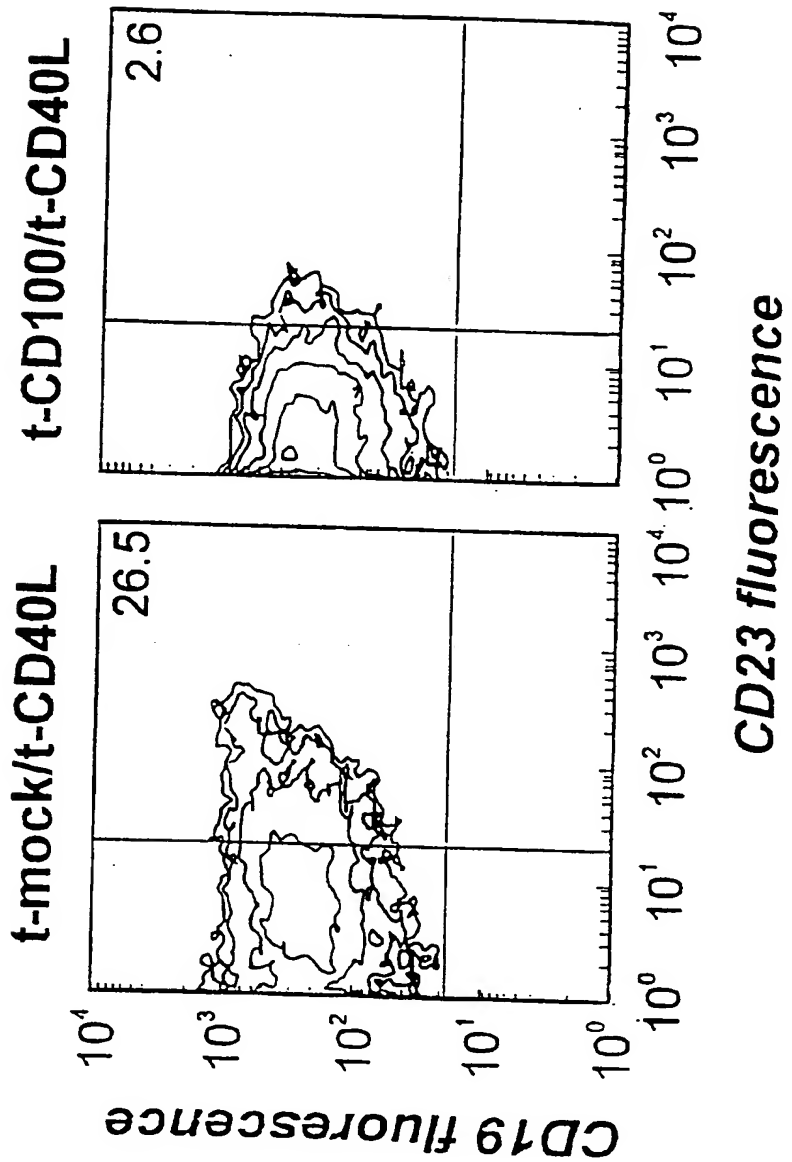


FIGURE 9

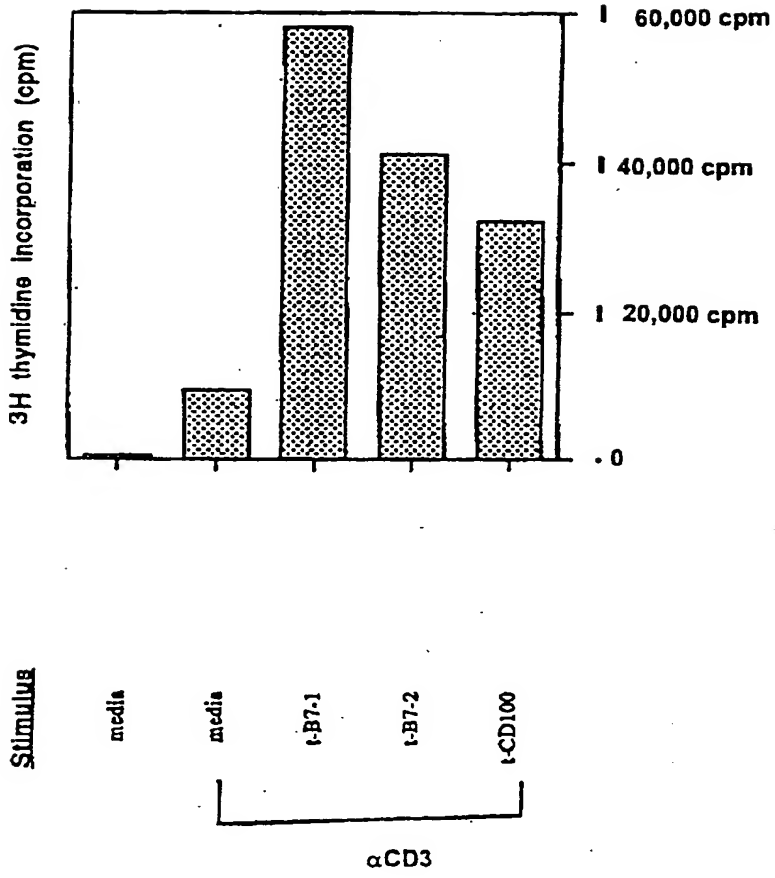


FIGURE 10

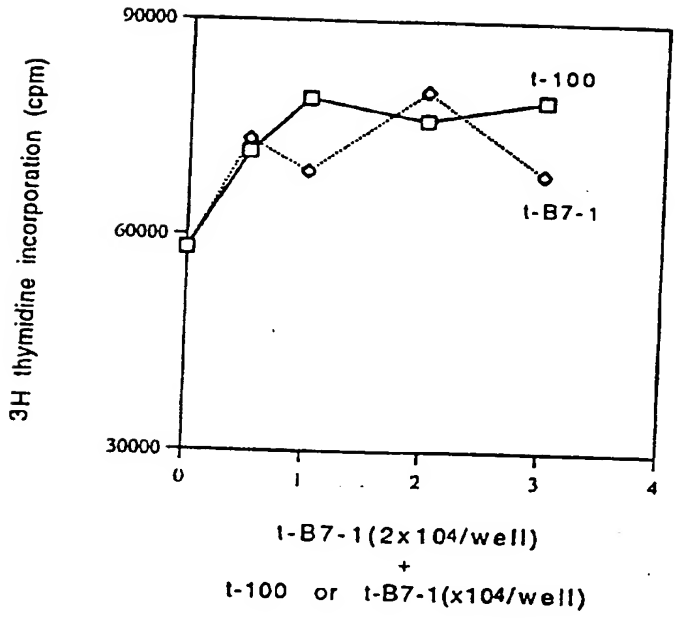


FIGURE 11

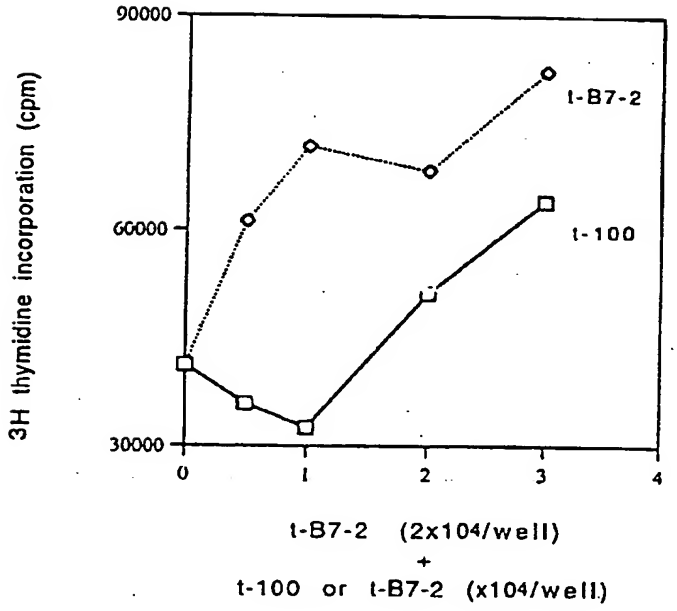


FIGURE 12

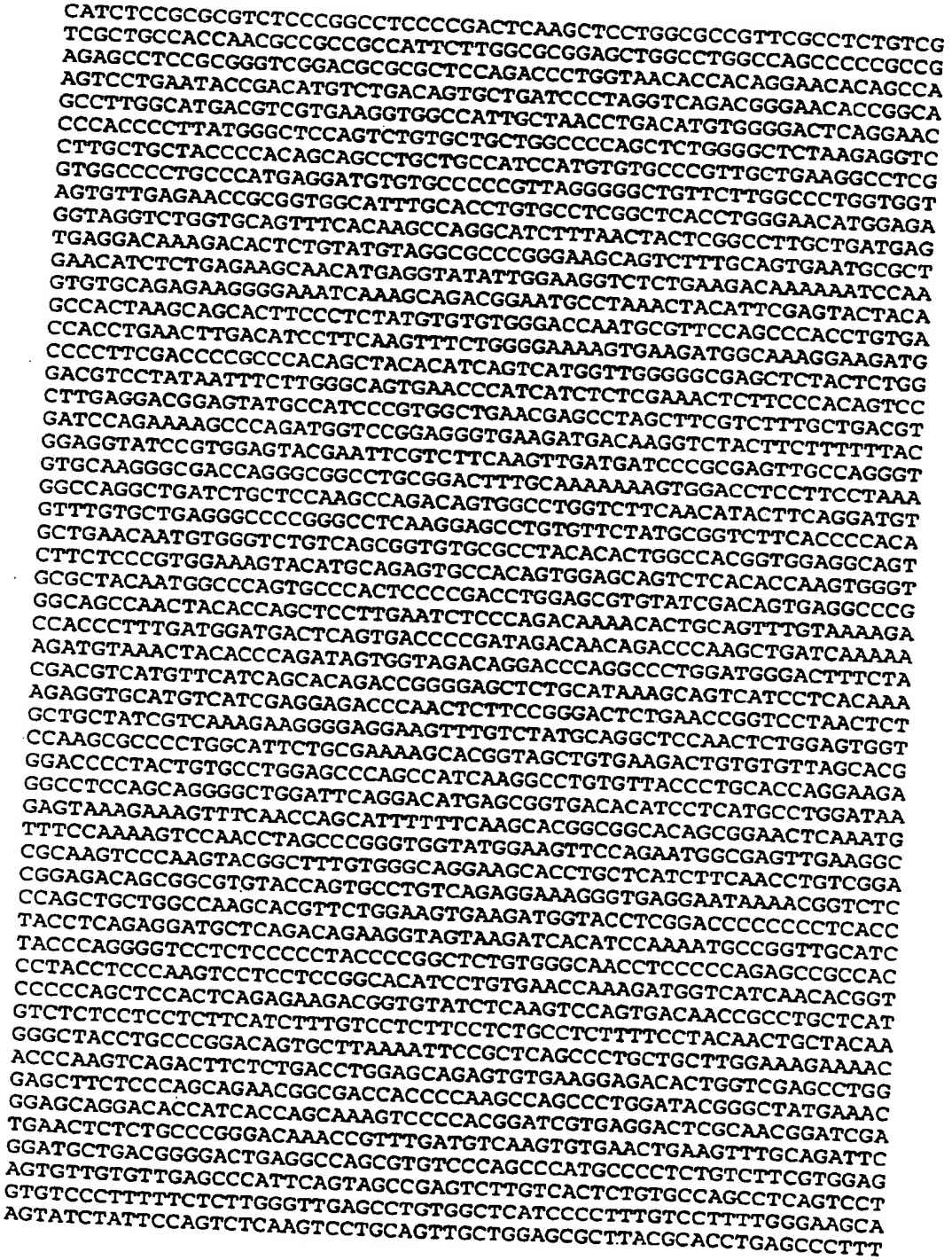


FIGURE 12 (CONTINUED)

GTGTCCTGGGGGAGAGATGGCCACCTCCGTGGGCTGCGAAGAGCCACCCCTTCCTCTTCC
GATTCTCCTAGCAGCCACTCAGAGATAATTTAATTCCAGATTGGAAACGCCCTTTTAGTT
TATCAGATTGGTAACTTACATCCTGCTGCCCAGATGGCACGGACAGTTTTCTTTCACTTA
ATTATTATTTTTTTTTTTAAGGATTTTCGCTCCTATTGTGTTGATGTCTTAGGTCATTTTC
TTTTTTTCTTTCTCTTTTTTTTATTACCAGAGGAGATGTTTTAATATTCATGAGAAGAGGA
ACATTTTCTAGATTTTTTTGTTGTTATATATTGAGATATAAAATATGGCTATGTTGCTTA
AGATTCTCAGGGATAGACTTATTTTTGTTAACTTCATTCTTTCCTGCTGTTAGGAACATA
GGCCTAAAATTGTCTCTTGAGTTTGCTCACCCTTTTGTTTTGGTAGGGTTTTTTTTGTTGT
TGCTGTTATTGTTTCTAGTTTTTAATCTTATTCATTTTGAAGGATTTTTCTTTCTGAACT
TTTTAAATTTTTATATTTTCCTGCCATACATCTACAAAGTGGGTTTTGAGTGAGGGCAGG
TGGCCCAGTGGCTTTGGGTGGCGACTGAGCTGGTCCCACGAGGGGAGGAGGGTTTATATA
CCCCATGACCCTGCGGCTTCTTGGCGCCTCCTGCCCATGAGGATCACATCCTGTCTCTCC
TTGCTTCCATCTCTCATCACTGCCCTTGGACTTCCGCCTTGACTGTCCATGAAAGACAGA
AATGGGTTGGGTAGTTGGGCTCCCAACCTCGGATGGTGACCGCAANATCCCGNGTGGGCG
GCCGGCCGNTCNTGCAGCCCGANTNTCCTGCCAGTGTNTTTCAGGATGTNAACGGGNGGT
ACGATTNTGGCATTTGTTTTTCGCTCNCCGNGNGTGGAANANTCATTCCATGTNGNGGGN
GACGAATTTTGGATTCCCCCCCACCCCCACCCCGNGCGGNGTAGANAATNNTTTTTTGCA
TGACANGATTTACCATTCGGTGTAAANATTTGNGTTTATAAGATTTANTTTGTTTTTATT
TTTTTANTNGGNACTGTANANATTTGNAAAGTACCCAAATAAACCNGNAGNTTTNTNAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

FIGURE 13

```
*** Aligned sequences:
C1 ( 1f): |>u 1>+++++ murineCD100 (4470 bases)++++++>u 4470>|
C2 ( 1f): |>u 88>++++++humanCD100coding region (2589 bases)++++++>u 2676>|

*** Alignment of first sequence with all others displayed
*** Key:
    UPPER CASE = aligned non-identical bases
    lower case = unaligned bases
    ---------- = aligned identical bases
    .......... = gap
```

```
mod2c-106 : GTGGCCCCTGCCCATGAGGATGTGTGCCCCCGTTAGGGGGCTGTTCTTGGCCCTGGTGGT
    cd100 : ............----------CA-----A----------C--A-------T-CA--

mod2c-106 : AGTGTTGAGAACCGCGGTGGCATTTGCACCTGTGCCTCGGCTCACCTGGGAACATGGAGA
    cd100 : GA----TG-G--A---A----------CA-A--C---A----------G--CA----

mod2c-106 : GGTAGGTCTGGTGCAGTTTCACAAGCCAGGCATCTTTAACTACTCGGCCTTGCTGATGAG
    cd100 : ---GCAC----------TG------A-----AC--------A---------C----

mod2c-106 : TGAGGACAAAGACACTCTGTATGTAGGCGCCCGGGAAGCAGTCTTTGCAGTGAATGCGCT
    cd100 : C--------G-----CT----CA----T--------G--G-----C--T-----C--A--

mod2c-106 : GAACATCTCTGAGAAGCAACATGAGGTATATTGGAAGGTCTCTGAAGACAAAAAATCCAA
    cd100 : C--------C--------G--------G----------A----------G-A--

mod2c-106 : GTGTGCAGAGAAGGGGAAATCAAAGCAGACGGAATGCCTAAACTACATTCGAGTACTACA
    cd100 : A--------A----------A-----A--G-----C--------C--G--G--G--

mod2c-106 : GCCACTAAGCAGCACTTCCCTCTATGTGTGTGGGACCAATGCGTTCCAGCCCACCTGTGA
    cd100 : ------C---GC---------T--C----------C--A--------GG-------

mod2c-106 : CCACCTGAACTTGACATCCTTCAAGTTTCTGGGGAAAAGTGAAGATGGCAAAGGAAGATG
    cd100 : ----------A--------T----------A--------------------

mod2c-106 : CCCCTTCGACCCCGCCCACAGCTACACATCAGTCATGGTTGGGGGCGAGCTCTACTCTGG
    cd100 : T-----T-----A--A----------C----------AT--A--A--T--T--G--
```

FIGURE 13 (CONTINUED)

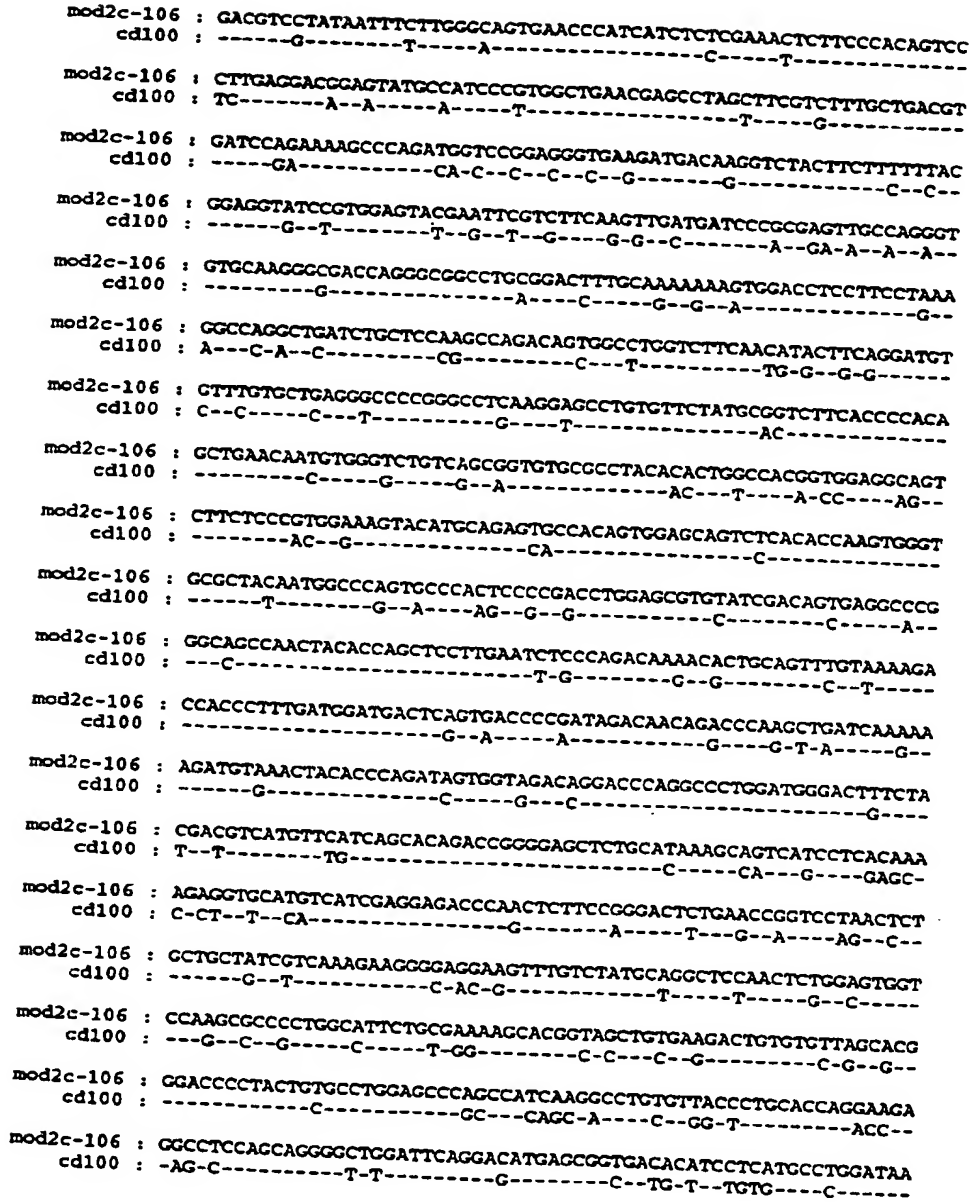


FIGURE 13 (CONTINUED)

```
mod2c-106 : GAGTAAAGAAAGTTTCAACCAGCATTTTTTCAAGCACGGCGGCACAGCGGAACTCAAATG
    cd100 : A-------G-----A-CGG--------------------T-------------G-----

mod2c-106 : TTTCCAAAAGTCCAACCTAGCCCGGGTGGTATGGAAGTTCCAGAATGGCGAGTTGAAGGC
    cd100 : C-C------A--------G--------CT-T-------------------T---------

mod2c-106 : CGCAAGTCCCAAGTACGGCTTTGTGGGCAGGAAGCACCTGCTCATCTTCAACCTGTCGGA
    cd100 : --AG--C-----------TC--A-------A--AA--T-------------T----A--

mod2c-106 : CGGAGACAGCGGCGTGTACCAGTGCCTGTCAGAGGAAAGGGTGAGGAATAAAACGGTCTC
    cd100 : A--------T--G----------------------G-----T-A---C----------T

mod2c-106 : CCAGCTGCTGGCCAAGCACGTTCTGGAAGTGAAGATGGTACCTCGGACCCCCCCCTCACC
    cd100 : ---AG--G-C-----------C-----------G----T--AAA-C--GTAGTGG-C--

mod2c-106 : TACCTCAGAGGATGCTCAGACAGAAGGTAGTAAGATCACATCCAAAATGCCGGTTGCATC
    cd100 : C----TGTCA-T--T-----------------G---TG-CA-----G--TT---G-----

mod2c-106 : TACCCAGGGGTCCTCTCCCCCTACCCCGGCTCTGTGGGCAACCTCCCCCAGAGCCGCCAC
    cd100 : C-----A-----T--------A-----A--CG--CA---C------T--G-G---AT---

mod2c-106 : CCTACCTCCCAAGTCCT....CCTCCGGCACATCCTGTGAACCAAAGATGGTCATCAACA
    cd100 : ---T---------.---gcgc--A-----------C----------C----------

mod2c-106 : CGGTCCCCCAGCTCCACTCAGAGAAGACGGTGTATCTCAAGTCCAGTGACAACCGCCTGC
    cd100 : ------------------G-----A--CA-------T--------C----------C-

mod2c-106 : TCATGTCTCTCCTCCTCTTCATCTTTGTCCTCTTCCTCTGCCTCTTTTCCTACAACTGCT
    cd100 : -------C---T--------T-------T------------------T----------

mod2c-106 : ACAAGGGCTACCTGCCCGGACAGTGCTTAAAATTCCGCTCAGCCCTGCTGCTTGGAAAGA
    cd100 : -T-----A---------A----------G----------G-----A--AA----G----

mod2c-106 : AAACACCCAAGTCAGACTTCTCTGACCTGGAGCAGAGTGTGAAGGAGACACTGGTCGAGC
    cd100 : -G-AG----------T----G-----GT--------CC----------GT-A--A----

mod2c-106 : CTGGGAGCTTCTCCCAGCAGAACGGCGACCACCCCAAGCCAGCCCTGGATACGGGCTATG
    cd100 : -A--------------------T--G--G--------------------C--C-------

mod2c-106 : AAACGGAGCAGGACACCATCACCAGCAAAGTCCCCACGGATCGTGAGGACTCGCAACGGA
    cd100 : -G--C-----A-----------------------------A-G--------A--GA---

mod2c-106 : TCGATGAACTCTCTGCCCGGGACAAACCGTTTGATGTCAAGTGTGAACTGAAGTTTGCAG
    cd100 : ----C--C--T------A-------G--C-----C----------G--------C--T-

mod2c-106 : ATTCGGATGCTGACGGGGACTGAGGCCAGCGTGTCCCAGCCCATGCCCCTCTGTCTTCGT
    cd100 : -C--A--C--A--T--A------.....................................
```